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Volume 41 October, 1951 Number 10

Phytopathology

AN INTERNATIONAL JOURNAL

Official Organ of

The American Phytopathological Society

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Subscription price: $10.00 per year, domestic and foreign

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph (d-2) Section 34.40, P. L. & R. of 1948. Authorized May 12, 1921.

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EFFECT OF HUMIDITY ON INFECTION OF TABLE GRAPES BY BOTRYTIS CINEREA

K. E. Nelson[1]

(Accepted for publication May 16, 1951)

Botrytis rot, caused by *Botrytis cinerea* Pers., is frequently a serious disease of table grapes in California vineyards if the fruit becomes wet. The free water on the fruit or the accompanying high relative humidity, or both, appear to be important factors contributing to the rapid development of this disease.

Du Plessis[2] concluded that outbreaks of botrytis rot were always preceded by rainfall and long periods of high relative humidity. Nelson[3] found that a wet period of 12 hr. at 12° to 20° C. resulted in appreciable infection even though the wet period was followed by a relatively dry one.

The significance of relative humidity during the incubation period for the amount of infection was shown subsequently when grapes were incubated in glass chambers. A constant flow of air was introduced into the chambers after it had been bubbled through water to increase the relative humidity (r. h.). Two lots of grapes which were dried after a ½-hr. wet period, then incubated 3 days, developed 94 and 97 per cent infected berries respectively.

In the present investigation all experiments were in a room maintained at 12° ± 0.5° C., and 87–90 per cent r. h. Air was pumped through tubing into the constant-temperature room at 2–5 lb. pressure per sq. in., then bubbled through pure water and different concentrations of calcium chloride-water solutions to adjust the water vapor content to the desired relative humidities before the air entered the respective incubation chambers.

The larger lots of grapes were incubated in 14-liter glass desiccators, capable of holding about 7 lb. of fruit; the smaller lots in 1-qt. chambers. The air entered at the bottom of each chamber, diffused upward between the berries, and escaped through an outlet at the top. A flow of 1–8 liters per min. was sufficient for complete replacement of air every 2–15 min. in the desiccators, and every 8–60 sec. in the smaller chambers.

The wet-bulb temperature depression was determined by means of thermocouples of No. 30 gauge iron-constantan wire connected to a Leeds and Northrup potentiometer, inserted into the small outlet of each chamber;

[1] Report of a study made under the Research and Marketing Act of 1946 while the writer was an Agent (Plant Pathologist) of the U. S. Department of Agriculture at the Horticultural Field Station, Fresno, California. Grateful acknowledgment is made to W. T. Pentzer and W. R. Barger; to W. S. Ballard (retired) of the U. S. Department of Agriculture; and to Wm. B. Hewitt of the University of California, for helpful suggestions during the course of this investigation.

[2] Du Plessis, S. J. Studies on the wastage of export grapes with special reference to that caused by Botrytis cinerea Pers. Dept. Agr. Union of S. Afr. Sci. Bul. 151. 1936.

[3] Nelson, K. E. Factors influencing the infection of table grapes by Botrytis cinerea (Pers.). Phytopath. 41: 319–326. 1951.

maximum depression was reached in less than 10 sec. in an air stream moving at less than 350 ft. per min. The relative humidity was then calculated from the Weather Bureau psychrometric tables.

The grapes were inoculated with conidia from 10-day-old cultures of *B. cinerea* grown on potato-dextrose agar at room temperature. The spore suspension was passed through fine muslin to strain out large mycelial fragments before it was atomized onto the fruit.

RELATIONSHIP OF WET PERIOD AND SUCCEEDING RELATIVE HUMIDITY

Four 6-lb. lots of Tokay grapes were rinsed with tap water, atomized with a spore suspension, and kept wet for 18 hr. Other lots were kept wet for 12, 6, 3, and 1 hr. The clusters were divided into bunches of 3 to 10 berries and then dried in an air blast for 1 to 2 hr. until no visible water remained. Each lot was placed in a chamber and incubated for 5 days at a given relative humidity, after which the percentage of infected berries was determined. Four levels of r. h. between 84 and 100 per cent were tested.

The length of the wet period following inoculation had no influence on the amount of infection which developed, but the greatest infection developed at the high r. h. values following the wet period.

Relative humidity lower than 84 per cent could not be maintained in the chambers because the fruit lost considerable moisture. There was always a relative humidity differential between inflowing and outflowing air of a chamber, and each air stream became more moist as the air diffused between the berries. The differential in the driest chamber was 24–30 per cent; it was 5–10 per cent in the next chamber, 7–8 per cent in the next, and 6–7 per cent in the most moist chamber.

Most of the infected berries in the lots incubated at the lower levels of r. h. appeared to have been invaded through the capstem area. It was possible that after the drying period free water may have remained unobserved in the crevices at the base of the capstem, in dead floral parts, or in the lenticels of the capstem itself. This would, in effect, prolong the wet period sufficiently to allow for lodged spores to germinate and infect the berries.

It is also possible that transpiration from the capstem may have produced enough moisture for the conidia to germinate, independently of an external water supply. Water loss through transpiration was indicated by the considerably higher r. h. of the outflowing than of the inflowing air of the chambers, even after several days' incubation; a longer time than would presumably have been required for residual water to evaporate completely.

The possibility that the capstem area was most susceptible to infection owing to more favorable moisture conditions, especially at lower humidity levels, was investigated with three lots of wet-inoculated Tokay grapes. The lots were kept wet 1 hr., dried 1 hr.; then incubated 5 days, one lot at

99 per cent, one at 97 per cent, and one at 90 per cent r.h. The infected berries of each lot were divided into two classes: those infected only through the capstem area, and those with lesions clearly not of capstem origin, i.e., infected through the skin of the berry.

At 99 per cent r. h., all berries were infected, 5 per cent through the capstem only. At 97 per cent r. h., 96 per cent infection occurred, 32 per cent through the capstem only. At 90 per cent r. h., 57 per cent infection occurred, 51 per cent through the capstem only.

Although 95 per cent of the berries incubated at 99 per cent r. h. had been infected through the skin, many had been invaded through the capstem area as well. At 97 per cent r. h., only about two thirds of the infected berries had skin infections, the other third being infected only through the capstem area. In the berries held at 90 per cent r. h., only about one-tenth had been infected through the skin, the capstem area being the chief court of infection. These results indicate that residual water in the capstem area or transpiration from the capstem tissue, or both, did, in effect, prolong the wet period.

To minimize the effect of residual water on infection and to reduce water loss from the berries, the technique was modified to use individual berries instead of small bunches. The capstem and adjacent area of each berry was coated with melted paraffin to a diameter of $\frac{1}{2}$ cm. Four lots of 3 lb. each were inoculated and kept wet for 6 hr. at 12° C., then dried and incubated for 5 days at r. h. levels of 94, 92, 90, and 80 per cent respectively. The corresponding infection percentages were 98, 88, 50, and 11.

From these results, it appears that at 12° C. and 80 per cent r. h. the conidia of Botrytis were not able to produce much infection. Even at 90 per cent r. h., infection was low. Above 90 per cent r. h., the fungus continued to grow after the drying period, and infection took place.

Each of five chambers was separated into quadrants so that four lots of about 150 berries each could be incubated simultaneously at the same relative humidity. The wet inoculations of three of the lots for each chamber were so timed that the wet period of each lot terminated at the same time. The lots were dried and three (each with a different-length wet period) were placed in each humidity chamber. A fourth lot in each chamber was subjected to no wet period, and was dusted rather than sprayed with conidia.

Infection of the dusted lots was moderate at 91 per cent r. h., high at 97 per cent, and complete at 99 per cent (Fig. 1). It was also moderate when wet periods of 18 and 24 hr. were followed by incubation at 62 per cent r. h., but much greater with incubation at 80 per cent r. h.; that for the shorter wet periods was proportional. Except for the 3-hr. wet-period lot incubated at 91 per cent r. h., the length of the wet period became less important in relation to the amount of infection as the incubation humidity became higher, until at 97 per cent r. h. it had no significance.

INFLUENCE OF HUMIDITY ON THE AMOUNT OF INFECTION OF DRY INOCULATED GRAPES

When there was no wet period, the critical humidity range was from about 90 to 97 per cent (Fig. 1). For more detailed analysis of this range, lots of 70 berries each were incubated in 1-qt. chambers. The recorded humidity of the outflowing air of each chamber was never more than 2 per cent greater than that of the inflowing air. In trial A of figure 2, four lots of Emperor grapes were dusted with conidia and incubated simultaneously at the indicated humidities. In trials B, C, and D, six lots were run at six different humidities.

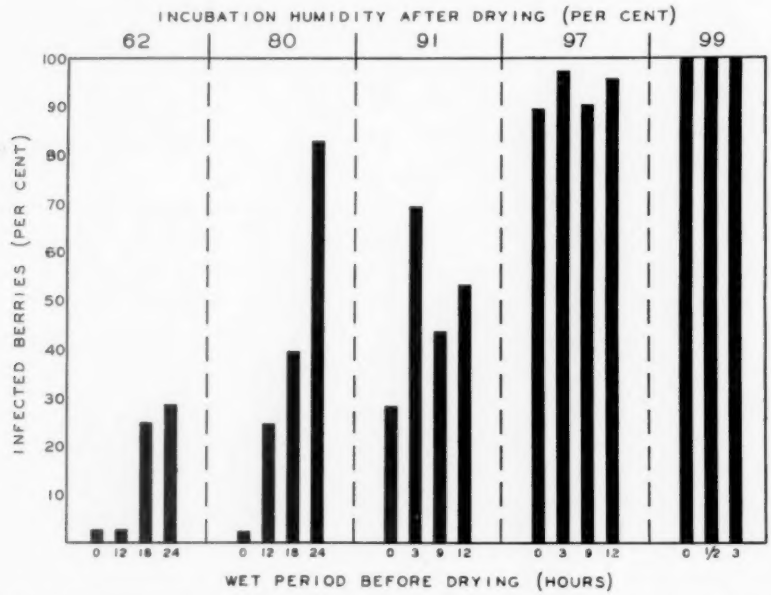

FIG. 1. Percentage of Botrytis infection of lots of Tokay grapes incubated and kept wet for 0 to 24 hr. and subsequently incubated 5 days at five different levels of relative humidity. Lots shown for 0 hours of wet period were dusted with conidia; other lots were sprayed with a spore suspension.

In all the trials, the amount of infection increased rapidly as the r. h. increased from 85 to 95 per cent. The lots held at 90 and 92 per cent r. h. in trial D show a lower infection percentage than do the corresponding lots of trials A, B, and C. The difference is exaggerated somewhat by the relatively lower incidence of infection in all six lots of trial D.

The critical range of 85 to 95 per cent r. h. in relation to the infection trend supports the work of Snow,[4] who was unable to germinate conidia of *B. cinerea* at 25° C. and r. h. levels below 93 per cent. The high incidence of infection in grapes dusted with conidia and incubated at r. h. levels as low as 88 per cent may be explained by temperature. According to

[4] Snow, D. The germination of mould spores at controlled humidities. Ann. Appl. Biol. 36: 1–13. 1949.

Wilson,[5] the saturation deficit expressed as partial pressure of water vapor is the same at 25° C. and 95 per cent r. h. as it is at 10° C. and 87.1 per cent r. h. (1.19 mm Hg.). On the basis of this statement, infection could be expected at an r. h. level as low as 88 per cent and at 12° C., if the maximum saturation deficit permissible for spore germination is the same for each temperature.

It is possible that the r. h. of the air on the grape surface was greater than that measured at the outlet of the chamber, because of loss of water from the fruit. Since the capstems were waxed, this moisture loss must have occurred through the skin. If the r. h. level at the skin surface was 5 per cent higher than that at the chamber outlet, this would explain spore germination (as indicated by some infection) in lots incubated at recorded r. h. levels as low as 85 per cent.

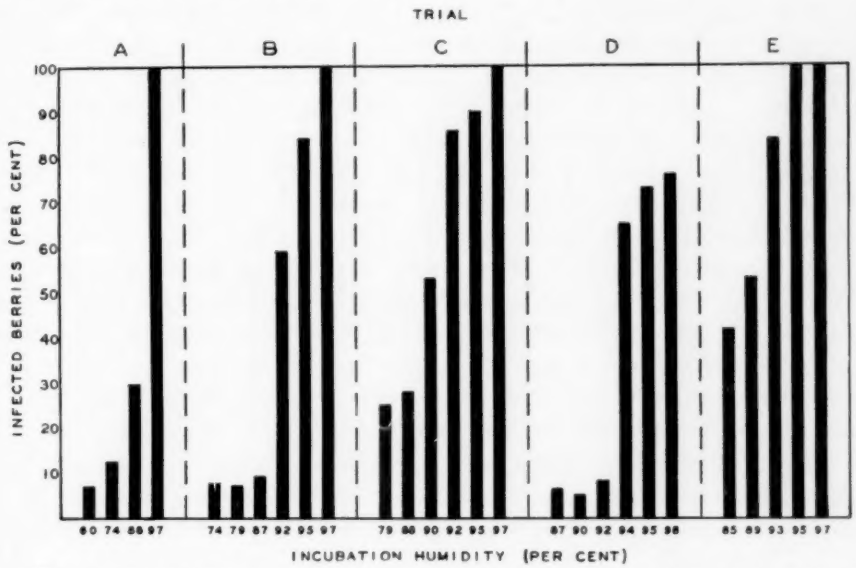

FIG. 2. Percentage of Botrytis-infected table grapes, when inoculated with dry conidia and incubated at different levels of relative humidity. Trials A, B, C, and D were with Emperor grapes, trial E with Tokay grapes.

Microscopic studies of spore germination supported the infection-trend results. At the time the lots of trials B and C (Fig. 2) were dusted with conidia, several clean glass slides were also dusted, and one was placed with each lot of grapes. After 48 hr. of incubation, a slide and two berries were removed from each humidity chamber. The spores on the slides were stained with cotton blue and examined for germination. A small square of skin was peeled from each berry, and similarly stained and examined. In two trials, consistent germination had occurred on slides and skin at an r. h. level as low as 92 per cent—one spore on the grape skin had germinated at 90 per cent r. h.

[5] Wilson, A. R. The chocolate spot disease of beans (Vicia faba L.) caused by Botrytis cinerea Pers. Ann. Appl. Biol. 24: 258–288. 1937.

SUMMARY

The length of the wet period was not significant in the development of infection of California-grown table grapes by *B. cinerea,* when clusters were inoculated and kept wet for different periods at 12° C., then dried and incubated at r. h. levels above 94 per cent. Eighty-five to 100 per cent of the berries were infected whether the wet period was 1 or 18 hr. in length. At lower humidity levels, the wet period became increasingly significant.

At r. h. levels of 85 to 90 per cent, considerably more infection occurred through the capstem than through the skin of the berry. This was due either to invisible residual water around the capstem or rapid transpiration from that area which, in effect, lengthened the shorter wet periods.

The lots of individual berries with waxed capstems which were similarly inoculated and kept wet for 12 hr. developed 3 per cent infected berries when subsequently dried and incubated at 62 per cent r. h.; 25 per cent at 80 per cent r. h.; 53 per cent at 91 per cent r. h.; and 96 per cent at 97 per cent r. h. Check lots dusted with conidia and not moistened developed 3 per cent infected berries at 62 and 80 per cent r. h.; 28 per cent at 91 per cent r. h.; 90 per cent at 97 per cent r. h.; and 100 per cent at 99 per cent r. h. Other lots dusted with conidia showed a rapid increase in the amount of infection in the range of 89 to 95 per cent r. h.

Microscopic observations showed that conidia had germinated on glass slides and on skin of grapes incubated at 12° C. in r. h. levels as low as 92 per cent, and one conidium had germinated on the skin of a grape incubated at 90 per cent r. h.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES
U. S. DEPARTMENT OF AGRICULTURE
FRESNO, CALIFORNIA

THE RELATION OF SOUTHERN BEAN MOSAIC TO BLACK ROOT

G. H. Bridgmon and J. C. Walker

(Accepted for publication May 16, 1951)

Black root was first described by Jenkins (5, 6) in Georgia on a number of varieties and breeding progenies of bean. The symptoms included systemic necrosis, discoloration of pod sutures, and vascular discoloration of roots. Jenkins succeeded in reproducing the disease by hypodermic injections of juice from fresh, diseased pods. Zaumeyer and Thomas (11) noted that black root symptoms appeared occasionally on varieties resistant to bean virus 1 when they were inoculated mechanically with a plant extract containing a strain of that virus. Grogan and Walker (3) confirmed this observation and showed that the necrotic reactions were reproduced in a larger percentage of cases when plants of the resistant varieties were inoculated by means of approach grafts with susceptible plants, the latter being inoculated and allowed to become systemically infected after the graft union was complete. They interpreted the necrotic symptoms as a hypersensitive reaction of the resistant variety when the virus was introduced into the phloem of the resistant plant. They also showed that Idaho Refugee, U.S. No.5 Refugee, Sensation Refugee 1066, and Sensation Refugee 1071, all of which derived their resistance from Corbett Refugee, responded with a necrotic reaction to approach-graft inoculation with bean virus 1, whereas the varieties Robust and Michelite, also highly resistant to bean virus 1, did not so respond. Ali (1) showed that the resistant character of Robust was genetically distinct from that of Idaho Refugee and U.S. No.5 Refugee. Grogan and Walker (3) showed further that the hypersensitive reaction appeared in Sensation Refugee 1066 when the susceptible Michelite and Great Northern U.I.15, as susceptible members of grafts with Sensation Refugee 1066, were inoculated with a strain of bean virus 1 known as Burkholder's strain.

When Stringless Green Refugee was grafted with Striped Creaseback, McCaslan, or Potomac and inoculated with the common or the greasy pod strain of bean virus 1, mild, late-developing necrosis appeared in the three last-named varieties. The type of resistance in these varieties has not been determined. The hypersensitive reaction to bean virus 1 does not explain the reported occurrence of black-root symptoms on such varieties as Kentucky Wonder and Black Valentine which are susceptible to bean virus 1 and in such varieties as Michelite and Great Northern U.I.15, which are susceptible to Burkholder's strain of bean virus 1.

Zaumeyer and Harter (9), in their description of southern mosaic of bean incited by bean virus 4, reported that resistant varieties responded by formation of local lesions on inoculated leaves with no evidence of systemic infection whereas susceptible varieties showed no local lesions on inoculated leaves but became mottled and distorted in systemically infected tissues.

Moreover, some varieties were susceptible to bean virus 4 and resistant to bean virus 1, e.g., U.S. No.5 Refugee. The strain of Idaho Refugee in use in this study is resistant to bean virus 1 and gives only local lesions with bean virus 4, although the strain of this variety used by Zaumeyer and Harter (9) was heterozygous or a mixture of lines resistant and susceptible, respectively, to the latter virus. Another variety, Stringless Blue Lake, was reported by Zaumeyer and Harter (10) to develop stem necrosis and necrotic spots on systemically invaded leaves in plants inoculated with bean virus 4.

The present paper is a report of an investigation to determine whether black-root symptoms can be induced by bean virus 4 if the virus is introduced into the phloem of otherwise resistant plants by the approach-graft method.

METHODS AND MATERIALS

Stock cultures of bean virus 1 and bean virus 4 maintained in this laboratory were used. The identity of both viruses was checked by their reaction on a number of bean varieties and other hosts and by their physical properties. The stock culture of bean virus 1 was maintained in Stringless Green Refugee and that of bean virus 4 on Sensation Refugee 1066 and U.S. No.5 Refugee, the last two varieties being resistant to bean virus 1. All plants on which the stock cultures were maintained were grown in the greenhouse from virus-free seed. The experimental plants were usually grown at temperatures ranging from 24° to 28° C. in greenhouses which were frequently sprayed and fumigated for control of aphids and other insects. After a fine carborundum powder was sprinkled on the leaves, they were inoculated mechanically with a glass spatula and then thoroughly washed with distilled water. Approach-graft inoculations were made according to the method outlined by Grogan and Walker (3), except that $\frac{3}{4}$-in. electrician's rubber tape was used instead of raffia. This was easy to apply and provided a more flexible graft for stem growth.

EXPERIMENTAL RESULTS

Seeds of varieties resistant and susceptible to the virus concerned were planted in 6-in. pots and separated by shallow wood partitions. The grafts were made when the plants reached the 2-leaf stage and approximately 5 days were allowed for the graft to take; the susceptible plant of each pair was then inoculated mechanically with the virus.

In the six tests with bean virus 4, Idaho Refugee was used as the resistant variety and various other Refugee varieties were used as susceptible members of the graft pair (Table 1). Systemic symptoms typical of southern bean mosaic appeared on the susceptible plants. Symptoms in the resistant plants became evident as early as 9 days after inoculation and as late as the time when the pods were 2 to 3 in. long. They consisted of reddish-brown to black necrosis of stems and petioles, killing of the uppermost

TABLE 1.—*Reaction of young plants of Idaho Refugee and other bean varieties resistant to southern bean mosaic when inoculated with bean virus 4 by the approach-graft method*

Varieties	Number of grafts	Number of graft pairs with necrosis in the resistant member
Stringless Green Refugee—Idaho Refugee[a]	13	9
Sensation Refugee 1066—Idaho Refugee	9	5
Sensation Refugee 1071—Idaho Refugee	8	6
U.S. No.5 Refugee—Idaho Refugee	10	7
Corbett Refugee—Idaho Refugee	2	2
Wisconsin Refugee—Idaho Refugee	2	2
U.S. No.5 Refugee—Stringless Blue Lake	9	9
U.S. No.5 Refugee—Kentucky Wonder	10	10
U.S. No.5 Refugee—Red Mexican No.34	9	9
U.S. No.5 Refugee—Great Northern U.I.123	12	12
U.S. No.5 Refugee—Great Northern U.I.59	3	3

[a] The first variety of each pair listed was inoculated by rubbing the primary leaves with infective juice of bean virus 4.

growing point, netlike necrotic lesions on trifoliate leaves, and discoloration of the tap root, particularly in the region just outside the vascular bundles (Fig. 1).

It was not unusual to find a range in affected plants from those with an occasional leaf with necrotic lesions to those with severe stem and root necrosis resulting in death of the plants. When a plant which had only mild necrotic leaf symptoms was kept until the pods matured, the ripe seeds were often shriveled and discolored. Such seeds germinated well, however, and plants derived from them had no signs of the disease. The use of such Refugee varieties as Sensation Refugee 1066, Sensation Refugee 1071, and U.S. No.5 Refugee, which are resistant to bean virus 1, would seem to rule out the chances of contamination with that virus. Nevertheless, recovery was made to indicator plants for bean virus 4 and positive results were always obtained.

Noninoculated grafts between the same varieties remained healthy. When inoculations were made on the resistant plant of a pair, local lesions typical of those described by Zaumeyer and Harter (9) appeared on the inoculated leaves of the resistant plant. There was no evidence that the virus passed through the graft into the susceptible plant. When Idaho Refugee plants were inoculated by hypodermic injection of plant extract containing bean virus 4 into stems and petioles, necrosis similar to that which resulted with approach-graft inoculations extended systemically throughout the plants.

Fourteen plants of Idaho Refugee were grafted to a like number of Stringless Green Refugee and inoculations with virus 4 were made on the susceptible member of approach grafts when the plants were blooming and after some pods had developed. In eight of the resistant plants systemic necrosis appeared in the pods 3 weeks after inoculation (Fig. 2). Similar results were secured when the susceptible plants were from varieties Sen-

sation Refugee 1066 and U.S. No.5 Refugee, the use of the last two varieties having the obvious advantage of preventing contamination with bean virus 1 in the susceptible host. Typical black-root symptoms were produced in four of five trials in which bean virus 1 was inoculated at the early podding

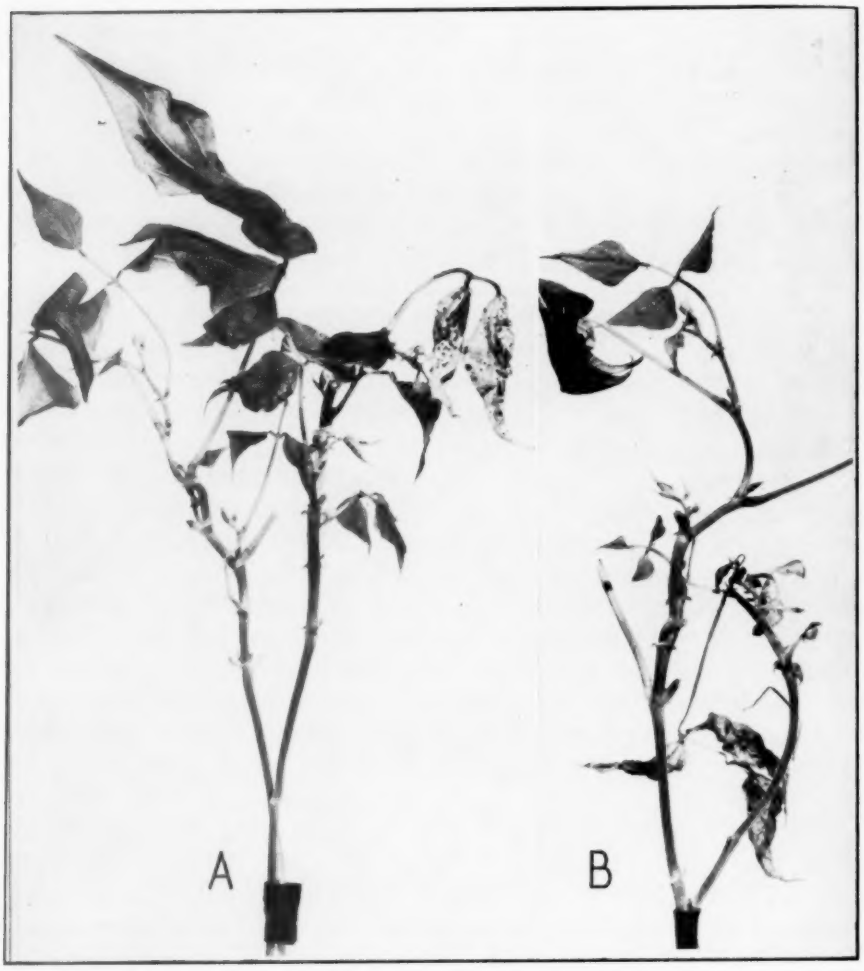

FIG. 1. Results of approach-graft inoculations in which the Stringless Green Refugee plant on the left of each pair was inoculated in the seedling stage with bean virus 4, resulting in typical mottle in the inoculated plant. The opposite plant in each pair is resistant Idaho Refugee. In A, systemic, necrotic lesions, top necrosis, and necrosis of stem and petiole appeared in the Idaho Refugee plant; in B, death of the resistant plant followed severe necrosis.

stage on plants of Stringless Green Refugee grafted to those of Idaho Refugee. Recovery to indicator plants from plants showing black-root symptoms on pods was successful with each virus when the test was made soon after the disease appeared. One successful maturing-plant graft gave numerous necrotic lesions on many trifoliate leaves but no stem, root, or pod

necrosis. A common type of necrosis in maturing resistant plants inoculated through approach grafts with bean virus 4 was development of a deep-seated lesion extending above or below the graft. Other common symptoms were the indiscriminate appearance of local lesions on systemi-

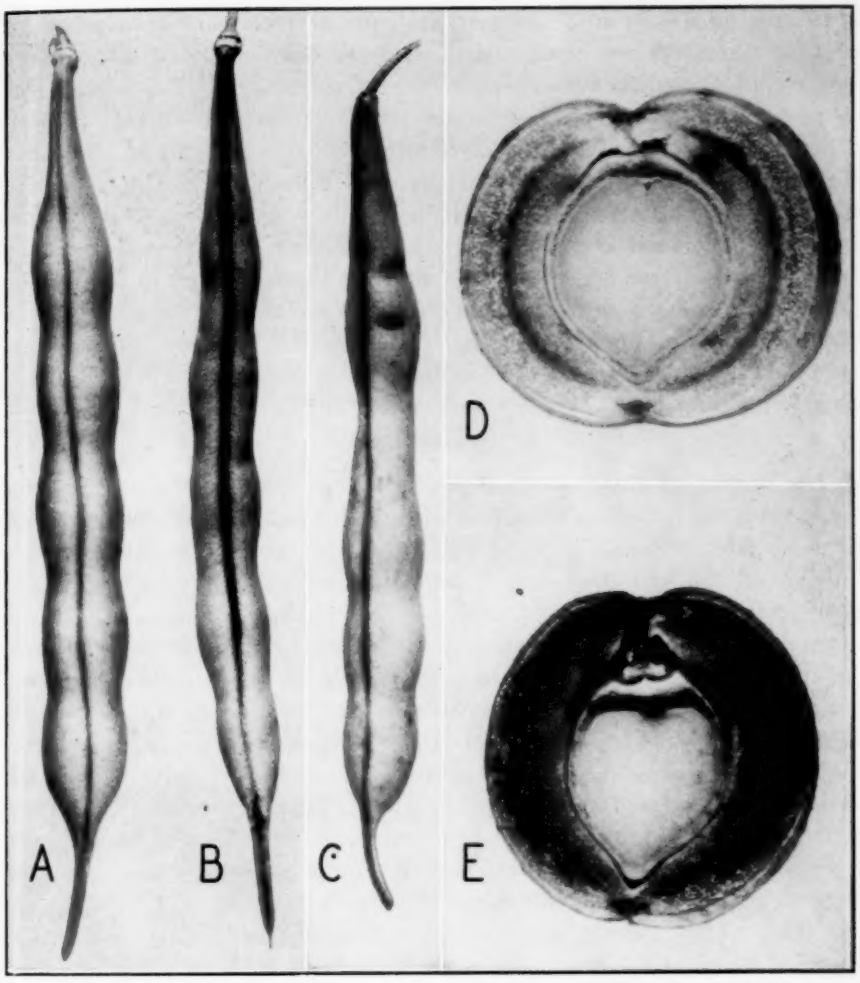

FIG. 2. Black-root symptoms on pods from resistant Idaho Refugee plants inoculated with bean virus 4 by the approach-graft method. A. Healthy pod. B, C. Infected pods showing dark sutures and necrotic spots. D. Cross section of healthy pod. E. Cross section of infected pod.

cally infected young trifoliate leaves usually on or adjacent to the veins, and occasional local lesions on systemically infected pods.

A parallel series of approach grafts between susceptible Stringless Green Refugee and four Refugee varieties resistant to bean virus 1 confirmed the early results of Grogan and Walker (3). The same range of disease symptoms was found in the resistant varieties as noted in the case

of bean virus 4 on Idaho Refugee. The symptoms were usually more severe than those incited by bean virus 4 and the necrotic lesions were gray to black in contrast with the reddish-brown to black lesions incited by that virus. Recovery to indicator plants for bean virus 1 indicated that the other virus was not present as a contaminant.

Grogan and Walker (3) secured systemic necrosis with bean virus 1 in varieties which derived their resistance from Corbett Refugee but not in Robust and Michelite, which was derived from Robust. Since black root has been reported on a number of varieties not related to Corbett Refugee, it was of interest to investigate varieties unrelated to the latter which give local-lesion response to rub-inoculation with bean virus 4. A number of such varieties and their response to inoculation with virus 4 by the approach-graft method are in table 1. It is evident from these results that many varieties which react to bean virus 4 by the formation of local lesions may develop systemic necrosis if the virus is introduced by the graft method. It is well to note here that Zaumeyer and Harter (10) secured some systemic necrosis with Stringless Blue Lake when inoculations were made by the rubbing method.

DISCUSSION

The results of this investigation show that black-root symptoms may result from the infection of certain varieties of beans with strains of bean virus 4. This is nearly a direct parallel to the results secured by Grogan and Walker (3) with bean virus 1. They found the hypersensitive reaction most pronounced in varieties derived from Corbett Refugee. Most such varieties are susceptible to southern bean mosaic. Idaho Refugee, the only variety of this group resistant to bean virus 4, is likewise the only one in which black root has been demonstrated by inoculation with this virus. Other varieties of beans such as Stringless Blue Lake, which is derived from White Creaseback (4, 7), and White-seeded Kentucky Wonder (U.S. No.3), which has as a parent the European variety, World Wonder (8), give the same type of necrotic reaction when inoculated with bean virus 4 through approach-grafts. In Stringless Blue Lake the symptoms are not unlike those described following rub-inoculation with bean virus 4 by Zaumeyer and Harter (10). Some of the varieties derived from Great Northern, the origin of which is unknown, also develop systemic necrosis when inoculated with bean virus 4. Thus the varieties resistant to bean virus 4 which show black root when inoculated with that virus by the approach-graft method come from various sources. Black root, when incited by bean virus 4, appears to be confined to those varieties which have a necrotic reaction to mechanical inoculation with the virus.

Since the symptoms of black root have been reported in the field on a large number of varieties, some of which do not contain resistance to bean virus 1 derived from Corbett Refugee, it would seem logical to assume that the necrotic reaction could well have been incited by bean virus 4. The

black root reported by Jenkins (5) in Georgia or by Blodgett and Schultz (2) in Idaho in such varieties as White-seeded Kentucky Wonder, Blue Lake, Great Northern, and Red Mexican might be explained as a hypersensitive reaction to the southern bean mosaic virus or some other virus rather than to the common bean mosaic virus.

SUMMARY

Many bean varieties with widely different sources of resistance to southern bean mosaic developed systemic necrosis when inoculated by the approach-graft method. The symptoms on the mature plants were similar to those described for black root. Varieties susceptible to southern bean mosaic were free from necrosis and had no symptoms of black root when inoculated in the same manner. Previous results on the production of black root by approach grafts between varieties resistant and susceptible to bean virus 1 were confirmed.

Necrosis incited by bean virus 4 was reddish-brown to black while that incited by bean virus 1 was gray to black. Necrosis incited by the former had a tendency to be milder than that of the latter.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. ALI, MOHAMED A. Genetics of resistance to the common bean mosaic virus (bean virus 1) in the bean (Phaseolus vulgaris L.). Phytopath. **40**: 69–79. 1950.
2. BLODGETT, EARLE C., and HERMAN K. SCHULTZ. Observations on unusual bean disease symptoms. U. S. Dept. Agr., Pl. Dis. Rptr. **29**: 429–432. 1945.
3. GROGAN, RAYMOND G., and J. C. WALKER. The relation of common mosaic to black root of bean. Jour. Agr. Res. [U. S.] **77**: 315–331. 1948.
4. JARVIS, C. D. American varieties of beans. N. Y. (Cornell) Agr. Expt. Sta. Bul. 260. 1908.
5. JENKINS, WILBERT A. A new virus disease of snap beans. Jour. Agr. Res. [U. S.] **60**: 279–288. 1940.
6. ———. A histological study of snap bean tissues affected with black root. Jour. Agr. Res. [U. S.] **62**: 683–690. 1941.
7. TRACY, W. W., JR. American varieties of garden beans. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 109. 1907.
8. WADE, B. L. Breeding and improvement of peas and beans. U. S. Dept. Agr. Yearbook 1937: 251–282. 1937.
9. ZAUMEYER, W. J., and L. L. HARTER. Two new virus diseases of beans. Jour. Agr. Res. [U. S.] **67**: 305–328. 1943.
10. ———, and ———. A severe necrosis caused by bean-mosaic virus 4 on beans. Phytopath. **34**: 510–512. 1944.
11. ———, and H. REX THOMAS. Shiny pod (greasy pod) virus and its identity with black root virus. (Abstr.) Phytopath. **38**: 29. 1948.

INFECTION OF SUGAR BEETS BY DITYLENCHUS DESTRUCTOR THORNE, THE POTATO ROT NEMATODE[1]

C. E. Dallimore and Gerald Thorne[2]

(Accepted for publication May 21, 1951)

The only hosts reported in the United States for the potato rot nematode, *Ditylenchus destructor* Thorne 1945, are potato tubers, *Solanum tuberosum* L., and dandelion, *Taraxacum officinale* Weber (12). When the effects of various cropping and cultural practices on the survival of *D. destructor* were studied, the infection in potatoes following 3 years of sugar beets, *Beta vulgaris* L., was nearly twice as great as that following 4 consecutive years of potatoes. Although infected sugar beets were not found during examinations of roots from a limited number of fields known to be infested, successful transfer from potatoes to sugar beets was accomplished under pot-culture conditions. A description of the symptoms is herein reported.

Vaňha and Stoklasa (13) recorded a Rübenfäule produced by *Tylenchus* n. sp., but they failed to assign a specific name. Ritzema Bos (9) gave a similar description of injury to mangels (*Beta vulgaris*) which he attributed to *Tylenchus dipsaci* (Kühn) Bastian. Goodey (2) also published a detailed description and illustrations of *T. dipsaci* attacking mangels. Roebuck and Hull (11) reported that sugar beets from 26 different growers of northern Lincolnshire were infected with *Anguillulina dipsaci* (Kühn) Ger. & Ben. Hastings and Newton (4) reported artificial transfers of *Ditylenchus dipsaci* (Kühn) Filipjev from red clover, *Trifolium pratense* L., and iris, *Iris xiphium* L., to sugar beets, but made their observations only on the seedling stage and therefore did not determine whether the colonies became permanently established. Other records are those of Goffart (1), Gram and Bovien (3), Kotthoff (5), van Poeteren (6, pp. 15, 16; 7, p. 17), Quanjer (8), and Ritzema Bos (10, p. 306). The characteristic symptoms described and illustrated by the various authors were very similar to those presented herein, and indicate that all were possibly dealing with *D. destructor*.

MATERIALS AND METHODS

Sugar-beet seeds were planted in 3-gal. glazed crocks filled with a sandy-loam soil and kept in the greenhouse until there was no danger of frost. By that time the beets had been thinned and were about $\frac{3}{4}$ in. in diameter. The crocks were then placed outdoors and soil was packed between them to prevent drying and too-sudden changes in temperature. Moisture requirements were supplied by hose irrigation.

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 330.

[2] Plant Pathologist, Idaho Crop Pest Control and Research Commission, Aberdeen, Idaho; and Senior Nematologist, Division of Nematology, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Salt Lake City, Utah.

Inoculations were made after the plants had become adjusted to the changed environment. A piece of potato tuber containing *D. destructor* was placed approximately 1½ in. below the surface of the soil and a short distance from the beets, or water containing *D. destructor* from potato tubers was poured over and around the beet crowns. As preparation for examining for the presence of *D. destructor,* the beets were carefully removed from the soil and washed; the roots were examined for lesions, and the washings from the beet roots and soil were screened and examined microscopically.

SYMPTOMS

Nemas enter the beets at, or near, the crown. The first evidence of their presence is a small discolored area beneath the cortex (Fig. 1, B) which

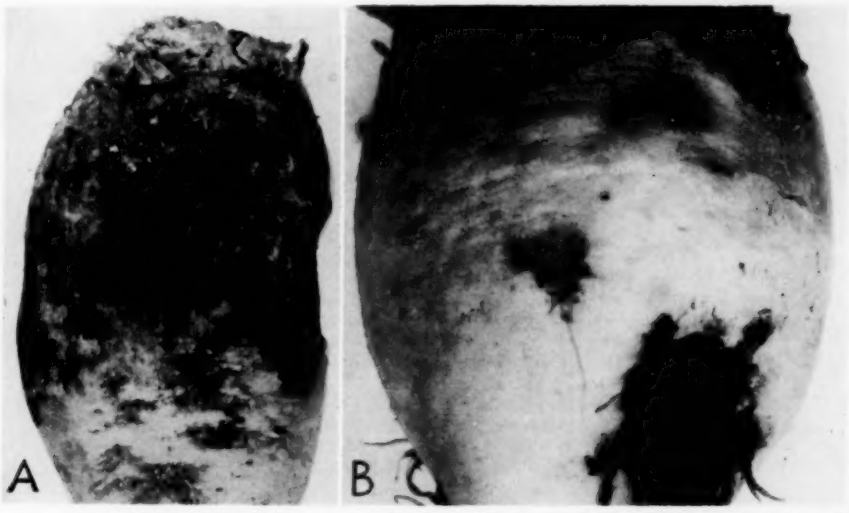

FIG. 1. External symptoms of infection of sugar beets caused by *Ditylenchus destructor:* A, advanced stage; B, small initial lesion.

rapidly develops until the entire crown may be involved (Fig. 1, A). Frequently the area branches downward and elongated dark welts form. Typically the injured tissues have a dry, honeycombed appearance, only the vascular fibers remaining intact. As infection progresses the cortex becomes brown and rugose and cracks may occur in the shrinking tissues. The nemas continue to advance into the healthy tissues and are followed by secondary fungi and bacteria, until eventually the entire beet is destroyed.

DISCUSSION

In the United States, *D. destructor* has been reported infecting potatoes on a few farms near Aberdeen, Idaho, but in only rare instances have the infections been severe enough to cause significant losses. Potatoes and sugar beets are the most common cultivated crops in this locality and the cropping system most generally followed consists of planting alfalfa with a

grain nurse crop. The alfalfa remains 3 or 4 years, is then plowed under, and is followed by potatoes for 1 or 2 years, after which sugar beets are planted a year or two, thereby completing the cycle. Thus the rotation includes from 2 to 4 years of host crops for *D. destructor*. Dandelions invariably are present in the old alfalfa stands and doubtless aid in carrying over the nemas between potato crops.

IDAHO CROP PEST CONTROL AND RESEARCH COMMISSION
ABERDEEN, IDAHO
AND
DIVISION OF NEMATOLOGY, U. S. DEPARTMENT OF AGRICULTURE
SALT LAKE CITY, UTAH

LITERATURE CITED

1. GOFFART, H. Über Schadauftreten von Stockälchen an Rüben und Sellerie. Deutsche Landwirtschaftliche Presse 62, Nr. 1. 1935.
2. GOODEY, T. The stem eelworm, Tylenchus dipsaci (Kühn 1857): Observations on its attacks on potatoes and mangolds with a host-list of plants parasitized by it. Jour. Helminth. **7**: 183–200. 1929.
3. GRAM, E., og P. BOVIEN. Rodfrugternes sygdomme og skadedyr. Det Kgl. Danske Landhusholdningsselskab. Copenhagen. 125 pp. 1944.
4. HASTINGS, R. J., and WM. NEWTON. Preliminary studies of the transfer of four strains of Ditylenchus dipsaci (Kühn 1858) Filipjev 1936. Can. Jour. Res. C, **15**: 168–174. 1937.
5. KOTTHOFF, P. Für Deutschland neue Wirtspflanzen der Anguillulina dipsaci (Kühn). Anzeiger für Schädlingskunde **13**: 60–63. 1937.
6. VAN POETEREN, N. Verslag over de werkzaamheden van den Plantenziektenkundigen Dienst in het jaar 1925. Versl. en Meded. Plantenziektenkundigen Dienst te Wageningen, 44. 124 pp. 1926.
7. ———. Verslag over de werkzaamheden van den Plantenziektenkundigen Dienst in het jaar 1926. Versl. en Meded. Plantenziektenkundigen Dienst te Wageningen, 51. 100 pp. 1928.
8. QUANJER, H. M. Een aaltjesziekte van de aardappelplant, de aantastingswijze en de herkomst van haar oorzaak, Tylenchus dipsaci Kühn. Tijdschr. over Plantenziekten **33**: 137–172. 1927.
9. RITZEMA BOS, J. Het stengelaaltje (Tylenchus devastatrix) oorzaak van "rot" in de bieten. Tijdschr. over Plantenziekten **14**: 65–77. 1908.
10. ———. Bekopte aanteekeningen op plantenziektenkundig gebied. Tijdschr. over Plantenziekten **33**: 296–314. 1927.
11. ROEBUCK, A., and R. HULL. The stem eelworm (Anguillulina dipsaci): attacks on sugar beet. Jour. Helminth. **15**: 243–245. 1937.
12. THORNE, GERALD. Ditylenchus destructor n. sp., the potato rot nematode, and Ditylenchus dipsaci (Kühn 1857) Filipjev 1936, the teasel nematode (Nematoda: Tylenchidae). Proc. Helminth. Soc. Washington **12**: 27–34. 1945.
13. VAŇHA, JOHANN, and JULIUS STOKLASA. Die Rüben-Nematoden (Heterodera, Dorylaimus und Tylenchus) mit Anhang über die Enchytraeiden. P. Parey, Berlin. 96 pp. 1896.

DIFFERENTIAL LETHAL EFFECT OF HOT-WATER TREATMENT ON THE LOOSE SMUT MYCELIUM IN NINE VARIETIES OF WINTER WHEAT[1]

WAYNE M. BEVER[2]

(Accepted for publication May 24, 1951)

Much has been written about the control of loose smut (*Ustilago tritici* (Pers.) Rostr.) of wheat by the hot-water method, but little has been published concerning the differential killing of the smut mycelium inside the seed of different winter wheat varieties. In the course of investigations on loose smut of winter wheats, it has been found that the lethal temperature for the smut mycelium inside the seed of wheat is not identical for all varieties or even within a close range. An experiment was set up to study this phenomenon and the results are reported here.

MATERIALS AND METHODS

Nine varieties of hard and soft red winter wheats were treated with hot water at seven different temperatures. Seven of these varieties were susceptible and two were resistant to the physiologic races of loose smut present during the investigation. The varieties used were: Kawvale (C.I.8180), Wisconsin Pedigree No. 2 (C.I.6683), Illinois selection 37-1146, Brill (C.I.11853), Royal (C.I.12558), Goens (C.I.4857), Wabash (C.I.11384), Clarkan (C.I.8858), and Saline (C.I.12674). All the seed used was naturally infected, having been produced in the physiologic-race–loose-smut nursery at Urbana, Illinois, where there was an abundance of inoculum. The only factors limiting infection were the inherent resistance of the variety and environmental conditions at the time of infection.

The seed was treated in 300-gm. lots in loosely woven cheesecloth bags at intervals of 3° from 129° to 147° F. The seed was presoaked in tap water for 4 hr. at 60° F. and then immersed in a constant-temperature hot-water bath at the treatment temperature. The temperature of the water bath dropped 2–3° immediately after immersion of the presoaked seed, but was restored within 1 to 2 min. and then maintained for 10 min., with a maximum variation of ± 0.5° F. The treated seed was then removed from the hot-water bath and cooled as quickly as possible by immersion in cold water. After the seed had cooled it was spread out on a greenhouse bench so as to dry thoroughly.

Germination tests of all treated seed and untreated checks were made in a sandbench in the greenhouse. Four hills, containing 30 seeds each from each treatment temperature and a nontreated control, were planted

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Illinois Agricultural Experiment Station.

[2] Agent (Pathologist), Division Cereal Crops and Diseases, and Associate Professor, Crop Pathology, University of Illinois, Urbana, Illinois.

in the field at the usual time for seeding winter wheat. Hand-threshed seed was used to avoid cracked or broken kernels, which are easily killed by high temperatures (5).

EXPERIMENTAL RESULTS

Effect of the hot-water heat treatment temperatures on seed germination. Treatment temperatures up to 132° F. had little effect on the percentage of seed germination (Table 1). In fact, with most varieties the

TABLE 1.—*Effect of temperature treatment on the germination of 4-hr. presoaked seed of nine varieties of winter wheat (4-yr. average)*

Variety	No treatment	Percentage germination at treatment temperatures of:						
		129° F.	132° F.	135° F.	138° F.	141° F.	144° F.	147° F.
Ill. Sel. 37–1146	90	95	90	59	32	51	30	17
Kawvale	80	89	85	57	33	44	13	8
Wisconsin Pedigree No. 2	90	87	85	55	28	45	20	1
Brill	86	89	82	62	55	44	46	8
Royal	95	93	79	59	58	40	24	10
Goens	91	92	95	71	47	47	31	19
Saline	91	92	96	72	41	54	47	29
Clarkan	86	86	83	75	45	49	43	36
Wabash	83	88	90	78	59	57	35	22

percentage of germination was higher for the seed treated at 129° F. than for the untreated check. Although not shown in table 1, the rate of germination was delayed in proportion to the treatment temperature; i.e., the higher the temperature, the slower the germination. However, with ample time, the total germination was not lowered to any great extent. The 138° F. temperature seemed to be a critical one for Illinois 37-1146, Kawvale, and Wisconsin Pedigree No. 2 varieties since it reduced their germination markedly, and each year the mature plants of these varieties were 4–6 in. shorter than those from seed treated at any of the other temperatures. Treatment of these varieties at 141° F. was less injurious to germination than that at 138° F.

The data in table 1 are of special interest in view of published results on the effect of the modified hot-water treatment on seed germination (1, 2, 3, 4, 5).

Temperature studies. Differences in lethal effect of the treatment temperatures on the loose smut mycelium inside the wheat seed are in table 2. In addition to these experiments a 20-bu. lot of Brill wheat was treated for a farmer-producer of certified seed. The percentage of smut in that lot was reduced from 6.5 to 2, but a retreatment at 132° F. eliminated the smut.

The temperature requirement for effective killing of the loose smut mycelium within the seed also varies with the variety (Table 2). Thus the lethal temperature for the smut mycelium was lower in Brill than in any other of the susceptible varieties, but the highest temperature, 147° F.,

TABLE 2.—*Effect of the hot-water treatments of nine varieties of winter wheat on the loose smut mycelium (4-yr. average)*

Variety	No treatment	Percentage of smutted heads in plants from seed hot-water treated at:						
		129° F.	132° F.	135° F.	138° F.	141° F.	144° F.	147° F.
Ill. Sel. 37–1146	2.75	1.25	2.25	1.25	0.75	0.2	0.0	0.0
Kawvale[a]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wisconsin[a] Pedigree No. 2	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brill	3.12	2.75	1.25	.25	0.0	0.0	0.0	0.0
Royal	3.92	3.92	2.50	1.00	1.75	0.0	0.0	0.0
Goens	2.70	1.00	1.95	1.00	1.25	0.25	0.0	0.0
Saline	2.50	2.50	2.25	1.25	2.50	0.75	0.0	0.0
Clarkan	6.75	4.25	5.62	4.00	3.25	4.25	3.25	1.37
Wabash	5.49	5.12	2.50	3.00	2.50	1.75	0.75	0.0

[a] Resistant to the physiologic races of loose smut present during the course of this investigation.

failed to kill all the smut mycelia in Clarkan. In one year the percentage of smut in Clarkan was the same at 147° F. treatment as for the control. With certain varieties in some years there was no smut from seed treated at the lower temperatures, whereas a small percentage of smut survived some of the higher temperatures. In other cases no smut developed after treatment at temperatures above 129° F. The reason for this is not definitely known, but conjecture would suggest either chance or the effects of maturation on the seed or smut mycelium.

Some attempt was made to determine the reason for the different temperatures necessary to kill the smut mycelium within the different varieties. It was thought that the amount of water absorbed during the 4-hr. presoaking might have some effect on the initial physiological activity of the smut mycelium, and that mycelium in a highly physiologically active condition is more susceptible to injury from high temperatures than when it is less active or completely dormant. Moisture tests of the seed before and after a 4-hr. presoaking were made to determine the amount of water absorbed by four representative varieties. At the end of the 4-hr. presoaking at 60° F. the seed was drained and surface-dried for 1 hr. Moisture determinations were by the oven method.

The results (Table 3) indicate that there is considerable difference in the amount of water absorbed by seed of the different wheat varieties. Brill absorbed the most water and Clarkan the least. Since the smut mycelium in Brill was killed at 138° F., whereas it survived 147° F. in Clarkan, it seems that the amount of water absorbed by the seed may determine the temperature necessary for killing the smut mycelium.

Effect of a longer presoaking and increase in treatment temperature on control of loose smut. As indicated in the foregoing, the old modified hot-water treatment for the control of loose smut in winter wheat is not completely effective. Also the temperature required to kill the smut mycelium within the seed is not the same for all varieties when a 4-hr. presoaking is

TABLE 3.—*Water absorption of seed of four winter wheat varieties during a 4-hr. presoaking*

Variety	Moisture content of seed expressed in percentage of dry weight		
	Before immersion	After 4-hr. presoak (10 replications)	Increase in moisture content
Hard seed type			
Wisconsin Pedigree No. 2	9.0	10.95	17.8
Brill	7.6	11.85	35.8
Soft seed type			
Saline	9.2	11.75	21.7
Clarkan	9.7	11.80	17.0

employed (Table 2). Obviously, therefore, further studies were necessary to discover an effective control and from the results in table 3 a longer presoaking seemed advisable, because of the significant differences between varieties in the amount of water absorbed in the 4-hr. presoaking.

Seed of representative varieties was selected and divided into two lots and treated as previously described, except that one lot was presoaked for 5 hr. and the other for 6 hr., and the treatment temperature was 130° F. instead of 129° F. The presoaked seed was immersed when the water temperature was 131 ± 5° F. On immersion, this temperature dropped 2–3° but was brought back to 130° F. as soon as possible (1½–2 min.). At the end of 10 min. from original immersion, the seed was taken out of the water bath, cooled immediately, and then dried.

No attempt was made to regulate the temperature of the water for the presoaking, primarily because under farm conditions equipment is not readily available for maintaining a temperature higher than the temperature of ordinary tap or well water.

Results for the 3-yr. test are in table 4.

TABLE 4.—*Result of a 5- and 6-hr. presoaking on effectiveness of hot-water treatment for control of loose smut; average of 3 years*

Variety	Percentage seed germination and percentage smut							
	Check; no treatment		No presoaking[a]		5-hr. presoaking		6-hr. presoaking	
	Germ.	Smut	Germ.	Smut	Germ.	Smut	Germ.	Smut
Ill. 37-1146-3	87	5.0	78	2	74	0.45	80	0
Brill	87	6.0	79	4	85	0.0	81	0
Goens	85	7.5	70	5	76	1.0	73	0
Clarkan	90	7.0	82	2	88	1.0	85	0

[a] No presoaking, but otherwise the treatment was the same.

The 5-hr. presoaking was not sufficient to eliminate all the smut in all varieties (Table 4), but the 6-hr. presoaking was completely effective for the varieties used in this experiment. In addition to the above, a 50-bu. lot of Royal, two 60-bu. lots of Seneca, a 3-bu. lot of Prairie, and a number

of small samples of hybrid material have been treated by this method with almost equal success. For the past 2 years, in treating the larger lots of seed, hot water has been used rather than live steam to maintain the desired treatment temperatures. Hot water seems to have a definite advantage over live steam in maintaining a constant temperature during the treatment period.

SUMMARY

Evidence indicates that the loose smut mycelium within the seed of different winter wheat varieties is not killed by identical treatment temperatures following a 4-hr. presoaking. Under these conditions the differing rates of water absorption among seeds of various varieties may determine the effectiveness of a particular treatment temperature.

A 6-hr. presoaking with a treatment temperature of 130° F. for 10 min. completely eliminated the smut infection in all varieties of wheat used.

Hot water, rather than live steam, introduced into the treatment container was more effective in maintaining a uniform temperature during the treatment period.

U. S. DEPARTMENT OF AGRICULTURE
AND
ILLINOIS AGRICULTURAL EXPERIMENT STATION
URBANA, ILLINOIS

LITERATURE CITED

1. APPEL, O. Theorie und Praxis der Bekampfung von Ustilago tritici und Ustilago nuda. Ber. Deut. Bot. Gesell. **27**: 606–670. 1909.
2. ————, and E. RIEHM. Die Bekampfung des Flugbrandes von Weizen und Gerste. Arb. K. Biol. Anat. Land- u. Forstw. **8**: 343–426. 1911. (A translation by V. F. Tapke.)
3. FREEMAN, E. M., and EDWARD C. JOHNSON. The loose smuts of barley and wheat. U. S. Dept. Agr., Bur. Plant Indus. Bul. 152. 1909.
4. KELLERMAN, W. A., and W. T. SWINGLE. Report on the loose smuts of cereals. Kans. Agr. Exp. Sta. Rpt. 2: 213–288. 1890.
5. TAPKE, V. F. Effects of the modified hot-water treatment in germination, growth, and yield of wheat. Jour. Agr. Res. [U. S.] **28**: 79–97. 1924.

PSEUDOMONAS MARGINALIS AS THE CAUSE OF SOFT ROT OF IMPORTED WITLOOF CHICORY

B. A. FRIEDMAN[1]

(Accepted for publication May 29, 1951)

Witloof chicory (*Cichorium intybus* L.), also known on the market as witloof, Belgian endive, or French endive, is imported from Belgium in sizable quantities each year. During the past ten years the most serious disease found on this vegetable on arrival at the New York market has been a bacterial soft rot. It has been commonly assumed that the causative agent is *Erwinia carotovora* (Jones) Holland. During 1949 a number of pathogenic bacteria were isolated from decayed witloof chicory leaves. Preliminary studies showed that all of these bacteria belonged to the genus *Pseudomonas* and that none were *Erwinia carotovora* or related species (9). Evidence obtained from comparative studies as to the identity of the witloof chicory bacterium with *Pseudomonas marginalis* (Brown) Stevens is presented in this paper, together with the results of studies on the effects of injury and temperature upon the development of decay on witloof chicory and on the infection of other vegetables by these bacteria.

DESCRIPTION OF DISEASE

Bacterial soft rot of witloof chicory appears as small, light brownish-vinaceous to deep brownish-vinaceous lesions[2] starting usually at the midribs of outer leaves which have been rubbed, bruised, or crushed. Often the rot is seen first on the leaf margins and tips, but occasionally it starts at the base of the head and progresses into the center. In early stages of the rot, affected tissues are soft and swarm with bacteria. As the lesions become older the affected tissues darken to a clay or sayal brown. The decay advances most rapidly under warm and humid conditions, the color deepens to a cinnamon-brown, russet, or argus brown, and the whole head becomes a slimy, foul-smelling mass of disintegrated tissue (Fig. 1). The decay may be confused macroscopically only with very young lesions of watery soft rot caused by species of *Sclerotinia*, but the presence of hyphae in the tissues examined microscopically distinguishes the decay caused by the fungi.

PATHOLOGICAL STUDIES

During the winter and spring of 1949 a number of strains of bacteria were isolated from decayed witloof chicory leaves. Four of these isolates produced a typical soft rot when inoculated into fresh leaves. The bacteria were reisolated in each instance. Subsequently the four original isolates and the four reisolates were purified eight times by growing single colonies on nutrient agar streak plates, and the pathological, morphological, and

[1] Associate Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

[2] Color descriptions based on Ridgway (17).

physiological characteristics of these eight phytopathogenic cultures were studied.

The necessity of wounding before infection would occur was demonstrated by the following inoculation experiment. Four uninjured witloof chicory leaves were carefully smeared with a virulent culture, a number of other leaves were inoculated by needle puncture with the same virulent culture, and still other leaves were punctured with a sterile needle. The leaves were placed in 1-qt. glass jars lined with wet paper and held at about 75° F. After 24 hr. extensive lesions had developed on the leaves inoculated with

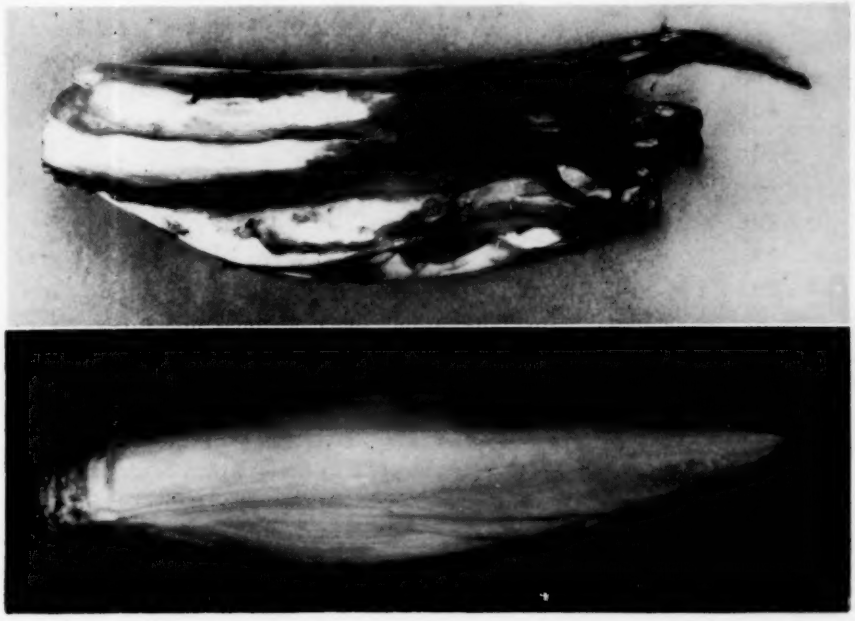

Fig 1. Soft rot of witloof; above, naturally infected head; below, healthy head.

bacteria by needle puncture, but after 48 hr. none had developed where the bacteria had been placed on unwounded tissues. Leaves punctured by a sterile needle were also free from decay. When the uninjured leaves which had been smeared with bacteria were punctured with a sterile needle at the site where the bacteria had previously been placed and were returned to the jars, lesions developed in 24 hr. This experiment was repeated on one other occasion, with similar results. Under the conditions of the test, the organism from witloof chicory apparently required injuries in order to cause decay.

To determine the effect of temperature upon decay development, two heads of witloof chicory were held overnight at each of eight temperatures —36°, 40°, 48°, 66°, 75°, 80°, 85°, and 89° F.—so that the leaves would assume the temperature at which they were to be incubated later. The following morning four leaves were removed from the head incubated at

each of the various temperatures and were inoculated by needle puncture with bacteria from a young culture of a highly virulent isolate from witloof chicory. A similar set of leaves from the second group of heads was inoculated with a virulent culture of *Erwinia carotovora*, originally isolated from a soft rot of potato tubers. Lengths of leaf lesions caused by each organism at the different temperatures were measured. The experiment was repeated at a later date. Data from the two experiments are in figure 2, where each point represents the average length of eight lesions. Both

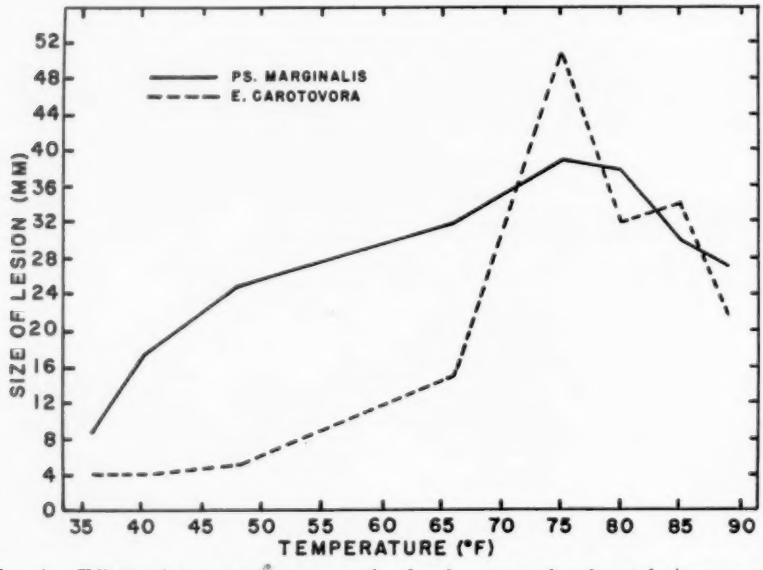

FIG. 2. Effect of temperature upon the development of soft-rot lesions on witloof chicory leaves. Measurements made 3 days after inoculation with *Ps. marginalis* and *Erwinia carotovora*.

organisms caused appreciable decay over the entire range of temperatures. However, the bacteria isolated from witloof chicory caused much more decay at 36°, 40°, 48°, and 66° than did *Erwinia carotovora*. The fact that *Ps. marginalis* caused decay at low temperatures may explain why considerable decay was frequently found in apparently well-refrigerated shipments.

Young cultures of the eight strains of bacteria from witloof chicory were used to inoculate washed, raw vegetables on two occasions, two inoculations being made each time. At the same time, control inoculations were made by means of a sterile needle. The vegetables were held in moist chambers at about 70° F. for 3 days and were observed daily. Between 19 and 22 of the 32 inoculations resulted in infections of carrot slices and of pea and broad-bean pods; more than 26 of the 32 inoculations of the remaining vegetables resulted in positive infections. Inoculations resulted in a soft rot of the following vegetables: witloof chicory (*Cichorium intybus* L.), escarole and endive leaves (broad- and curly-leaved types of *C.*

endivia L.), lettuce leaves (Iceberg and romaine varieties of *Lactuca sativa* L.), sliced cucumber fruit (*Cucumis sativus* L.), sliced onion bulbs (*Allium cepa* L.), sliced potato tubers (*Solanum tuberosum* L.), sliced carrot roots (*Daucus carota* L.), pea pods (*Pisum sativum* L.), string-bean pods (*Phaseolus vulgaris* L.), and broad-bean pods (*Vicia faba* L.).

MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES

Methods. Unless otherwise stated, standard methods were used to determine the morphological and physiological characteristics of the bacteria (20). Growth of the bacteria in relation to free oxygen was determined in broth cultures incubated in the presence of alkaline pyrogallol or underneath a thick layer of paraffin-petrolatum. Gray's method of staining flagella was used. Capsules were stained by Anthony's method, using 72-hr.-old growth from dextrose nutrient agar. Fluorescence was observed on nutrient agar and in Sullivan's asparagine broth, formula K (22, p. 129). Carbon compounds, at 1 per cent concentration, were sterilized by filtration and added aseptically to sterile synthetic broth containing brom cresol purple as an indicator. Adonitol and trehalose were used at 0.25 per cent concentration. One per cent Witte's peptone broth and litmus paper, as described by Brown in her study of *Ps. marginalis*, were used in some tests (2); in others an asparagine base medium (the agar and litmus omitted and brom cresol purple substituted) used by Stapp in tests on *Ps. intybi* was employed (21). Organic acids and sodium tartrate were tested at a 0.15 per cent concentration in synthetic broth with phenol red as an indicator.

Description of the causal organism. The bacteria isolated from witloof chicory soft rot are short, Gram-negative rods, 0.78–2.22 mu long by 0.48–0.79 mu wide (average 1.56 by 0.72 mu). The bacteria are motile, with one to seven, usually one to three, polar flagella. They form no spores, produce a thin capsule on dextrose nutrient agar, and occur mostly as single cells; but they are often in pairs and occasionally in short chains. On nutrient agar the colonies are white to creamy, circular, 3–4 mm. after 5 days at 70° F., smooth, homogeneous, slightly moist, not spreading, with center slightly raised, margin flattened, and edges minutely lobed; the medium is tinged by a green fluorescent pigment. The bacteria are strict aerobes and form a green fluorescent pigment but no pyocyanin. Gelatin is liquefied. Nitrates are rapidly reduced to nitrites without gas formation by four strains; one isolate and its reisolate produced gas; another isolate and its reisolate usually failed to reduce nitrates. Ammonia is formed, but not indol or hydrogen sulphide. Acid but no gas is formed in synthetic broth containing arabinose, xylose, dextrose, levulose, mannose, galactose, sucrose, lactose (weak reaction, occasionally negative), maltose (variable reaction, mostly weak, but often negative), trehalose, glycerol, mannitol, sorbitol, or inositol. No acid (occasionally a trace) is produced from raffinose or adonitol. An alkaline reaction is produced in broths containing acetic

acid, lactic acid, succinic acid, and citric acid; growth is poor or absent in broth containing sodium tartrate, and no alkali is formed. Starch is very weakly hydrolyzed. Brom cresol purple milk has an alkaline reaction and is peptonized with no coagulation. Growth takes place in nutrient broth containing 5–6 per cent, but not in that containing 7 per cent, NaCl. Optimum growth temperature is in the range 20°–30° C. (68°–86° F.). Growth at 37° C. (98.6° F.) is absent or slight. The thermal death point is about 51°–53° C. (123.8°–127.4° F.).

FLUORESCENT BACTERIA PREVIOUSLY REPORTED ON CICHORIUM SPP.

Swingle (23) in 1925 described *Pseudomonas intybi* (Swingle) Stapp and *Ps. cichorii* (Swingle) Stapp as the causes of a center rot of French endive (*Cichorium intybus*) in Montana. Kotte (10) found that *Ps. endiviae* Kotte caused a leaf spot and rot of *C. endivia* in Germany and that this organism was able to infect head lettuce inoculated artificially. Stapp (21) identified *Ps. intybi* from natural infections on *C. endivia* and *Lactuca sativa* and found that this organism was able to infect *C. intybus*. Okabe (15) found that a fluorescent organism, *Bacterium formosanum* Okabe, caused a soft rot of *C. intybus* in Formosa and infected a number of artificially inoculated plants including *Lactuca sativa, Solanum tuberosum, Lycopersicon esculentum* Mill., *Allium cepa, Nicotiana* sp., *Daucus carota,* and *Brassica* spp. Thornberry and Anderson (24) reported the presence of *Ps. cichorii* on *C. intybus* in Illinois. *Ps. marginalis,* which was originally described as causing a marginal leaf disease of lettuce (2), was found in natural infections on *C. intybus, C. endivia,* and *Lactuca sativa* in Argentina (14). Dowson (6) reported that *Ps. marginalis* isolated from lettuce in England can cause a soft rot of potato, onion, cucumber, and tomato.

At the present time, therefore, five species of fluorescent bacteria have been reported on *Cichorium* spp.: *Ps. intybi, Ps. cichorii, Ps. endiviae, Bact. formosanum,* and *Ps. marginalis.* Of these, *Ps. endiviae* and *Bact. formosanum* are considered probable synonyms of *Ps. cichorii* (1).

COMPARISON OF WITLOOF CHICORY SOFT-ROT BACTERIA WITH PSEUDOMONAS SPP. REPORTED ON SPECIES OF CICHORIUM

In laboratory tests the bacteria from witloof chicory differed from *Ps. cichorii* (culture PC1 received from W. H. Burkholder) in liquefying gelatin, in forming acid from sucrose and sorbitol, and in usually reducing nitrates. The bacteria from witloof chicory caused a soft rot of slices of potato, cucumber, and carrot, but Swingle (23) reported failure of *Ps. cichorii* to infect these. On the other hand, *Bact. formosanum,* which is stated to be a probable synonym of *Ps. cichorii* (1), was described as able to infect these plants (15).

A culture of *Ps. intybi* was not obtainable. The original description of this organism is meager (23). However, as described later (21), *Ps. intybi* differs from the witloof chicory bacteria in failure to ferment sucrose, in

coagulating but not clearing milk, and growing at 40°–42° C. (104°–107.6° F.). Both organisms cause a soft rot and infect chicory, endive, and lettuce. *Ps. intybi,* however, has been reported unable to infect beans, carrots, cucumbers, and potatoes (21, 23).

Two cultures of *Ps. marginalis* were received from W. J. Dowson: culture No. 136 isolated by him from lettuce, and No. 249 originally isolated by Miss Nellie Brown. The latter strain was described originally as forming acid in sucrose broth. It is no longer able to ferment this sugar, however, so that now the principal difference between these two cultures and those from witloof chicory is the ability of the latter to ferment sucrose (in both synthetic broth and Witte's peptone broth). Differences in the reactions of bacteria identified as *Ps. marginalis* have been reported. For example, nitrate reduction by *Ps. marginalis* has been reported as both positive (2, 7) and negative (4, 14). Acid formation from sucrose also has been stated to be positive (2, 14) and negative (4, 7, 18), and both the ability and the failure to coagulate milk have been reported (2, 4). In spite of variations in the physiological reactions of different isolates, soft-rot bacteria from witloof chicory are believed to belong to one species, and are considered to be similar to *Ps. marginalis* (Table 1).

TABLE 1.—*Comparison of witloof chicory bacteria with Pseudomonas marginalis*

Character	*Ps. marginalis*[a]	Witloof bacteria
Flagella	Bipolar, 1–3	Polar, 1–7, mostly 1–3
Size	0.83–1.87 × 0.42–0.83 mu	0.78–2.22 × 0.48–0.79 mu
Capsules	Present	Present
Spores	None	None
Gram stain	Negative	Negative
Green fluorescence	Positive	Positive
Colony color	White, creamy, to yellow	White to creamy
Growth in NaCl broth	6 per cent, none at 8 per cent	5–6 per cent, none at 7 per cent
Gelatin liquefaction	Positive	Positive
Indol production	None	None
Nitrate reduction	Positive	Positive
H_2S production	None	None
NH_3 production	Positive	Positive
Oxygen relationship	Aerobe	Aerobe
Fermentation:		
Witte's peptone		
Dextrose	Acid, no gas	Acid, no gas
Sucrose	do	do
Lactose	Alkaline, no gas	Alkaline, no gas
Maltose	do	do
Glycerol	do	do
Mannitol	do	do
Thermal death point	52°–53° F.	51°–53° F.
Optimum growth temperature	25°–26° F.	20°–30° F.[b]
Maximum growth temperature	38° F.	None or slight at 37° F.
Starch hydrolysis	Feeble	Very weak
Milk	Soft curd[c]	Not coagulated[d]

[a] As described by Brown (2).
[b] No effort was made to determine the optimum growth temperature more closely.
[c] Tested in litmus milk.
[d] Tested in brom cresol purple milk.

Ps. marginalis was described originally as causing a marginal leaf disease of greenhouse-grown lettuce, but it was not observed to cause a soft rot (2). Since the first description, however, organisms identified as *Ps. marginalis* have been reported to cause a soft rot of lettuce, endive, chicory, potato, onion, cucumber, and tomato (6, 14, 19).

DISCUSSION

In the original description, Brown (2) differentiated *Ps. marginalis* from another lettuce pathogen, *Ps. viridilivida* (Brown) Holland, which she had described previously. It has been reported several times that *Ps. marginalis* is identical with *Ps. aeruginosa* (Schroeter) Migula (8, 13, 16). However, it has been stated that the strains of *Ps. marginalis* which produce pyocyanin (in addition to fluorescein) may be considered to be *Ps. aeruginosa*, whereas other strains which produce fluorescein but not pyocyanin are called *Ps. marginalis* (7, 16, 18). Of the fluorescent bacteria studied in this laboratory, including the isolates from witloof chicory and *Ps. marginalis* (No. 136 and 249), only *Ps. aeruginosa* (ATCC 10145) produced pyocyanin. A number of other phytopathogenic bacteria have been compared with *Ps. marginalis* by various investigators. For example, *Ps. polycolor* Clara, *Ps. aeruginosa*, *Ps. marginalis*, and other species were said to be related (3, 8). *Ps. intybi* was said to differ in several characteristics from *Ps. marginalis* (21), and the latter organism was considered to be related to but distinct from *Ps. aptata* (Brown & Jamieson) Stevens (11, 16) and *Ps. syringae* van Hall (11). The characteristics of *Ps. desaiana* (Burkholder) Breed et al., *Ps. marginalis*, *Ps. aptata*, and *Ps. xanthochlora* (Schuster) Stapp were compared and the points of resemblance noted (5). Serologically, *Ps. marginalis* was considered distinct from *Ps. aptata*, *Ps. syringae*, *Ps. aeruginosa*, and other species (18).

It is evident from the preceding literature survey that *Ps. marginalis* is similar in many respects to several other phytopathogenic bacteria. This has been true in comparative physiological studies made in this laboratory. However, in agreement with other reports (1, 7, 12) this organism is considered to be a valid species.

Witloof chicory is shipped to the United States under refrigeration (about 36° F.) in order to retard decay development in transit. The graph (Fig. 2) shows that *Ps. marginalis* causes appreciable decay even at 36° F. but that it is more destructive at higher temperatures. Since the freezing point of this commodity is about 30.7° F. (25), a transit temperature of about 32° to 33° F. may be expected to reduce further the decay without at the same time endangering the commodity.

Most of the soft rot observed in commercial shipments seemed to be associated with leaf injuries. This fact, together with studies showing the necessity of wounding in order to inoculate leaves successfully, emphasizes the need of careful handling to avoid bruising of witloof chicory, so as to reduce rot in transit.

SUMMARY

A bacterial soft rot of imported witloof chicory is described. Lesions start at injuries. Decay lesions enlarge most rapidly at about 75° to 80° F., but appreciable rot occurs at 36° to 48° F. Careful handling to avoid bruising and a lower transit temperature (about 32°–33° F.) than now employed are suggested to reduce decay during transportation and marketing.

Morphological and physiological studies were made of a number of organisms isolated from infected witloof chicory leaves. A laboratory comparison of *Ps. cichorii* and two strains of *Ps. marginalis*, as well as the original description of the latter bacterium, indicates that the organism from witloof chicory is *Ps. marginalis* (Brown) Stevens.

Bacteria from witloof chicory caused a soft rot of inoculated lettuce, escarole, endive, cucumber, onion, potato, carrot, string bean, and broad bean.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES
U. S. DEPARTMENT OF AGRICULTURE
ROOM 1022, 641 WASHINGTON STREET
NEW YORK 14, NEW YORK

LITERATURE CITED

1. BREED, R. S., ET AL. Bergey's Manual of Determinative Bacteriology. 1529 pp. The Williams & Wilkins Co., Baltimore, Maryland. 1948.
2. BROWN, NELLIE A. Some bacterial diseases of lettuce. Jour. Agr. Res. [U.S.] **13**: 367–388. 1918.
3. CLARA, F. M. A new bacterial leaf disease of tobacco in the Philippines. Phytopath. **20**: 691–706. 1930.
4. ———. A comparative study of the green-fluorescent bacterial plant pathogens. Cornell Univ. Agr. Exp. Sta. Mem. 159. 1934.
5. DESAI, S. V. Stinking rot of sugar cane. Ind. Jour. Agr. Sci. **5**: 387–392. 1935.
6. DOWSON, W. J. The identification of the bacteria commonly causing soft rot in plants. Ann. Appl. Biol. **28**: 102–106. 1941.
7. ———. Manual of Bacterial Plant Diseases. 183 pp. A. & C. Black, London. 1949.
8. ELROD, R. P., and A. C. BRAUN. Pseudomonas aeruginosa: its role as a plant pathogen. Jour. Bact. **44**: 633–644. 1942.
9. FRIEDMAN, B. A. Soft rot of witloof chicory caused by Pseudomonas sp. (Abstr.). Phytopath. **40**: 9–10. 1950.
10. KOTTE, W. Eine bakterielle Blattfaule der Winter-Endivie (Cichorium endivia L.). Phytopath. Ztschr. **1**: 605–613. 1930.
11. LACEY, MARGARET S. Studies in bacteriosis. XIX, Part 2. Ann. Appl. Biol. **19**: 190–203. 1932.
12. MAGROU, J., *in* P. Hauduroy et al. Dictionaire des Bacteries Pathogenes pour l'Homme, les Animaux et les Plantes. 597 pp. Masson et Fils, Paris. 1937.
13. MEHTA, MANECK M., and EMILY M. BERRIDGE. Studies in bacteriosis. XII. B. pyocyaneous as a cause of disease in lettuce and the identity of B. marginale with this organism. Ann. Appl. Biol. **11**: 318–323. 1924.
14. MUNTANOLA, MARIA. Bacteriosis de las Hojas de Lechuga (Pseudomonas marginalis (Brown) Stapp). Minist. Agr., Buenos Aires, Argentine. 4th Year, No. 40, Series A, pp. 1–11. 1948.
15. OKABE, N. Bacterial diseases of plants occurring in Taiwan (Formosa). V. Jour. Soc. Trop. Agr. **7**: 57–66. 1935.
16. PAINE, S. G., and J. M. BRANFOOT. Studies in bacteriosis. XI. A bacterial disease of lettuce. Ann. Appl. Biol. **11**: 312–317. 1924.
17. RIDGWAY, R. Color Standards and Color Nomenclature. 43 pp. 53 plates. Pub. by the author, Washington, D. C. 1912.
18. ST. JOHN-BROOKS, R., ET AL. The investigation of phytopathogenic bacteria by serological and biochemical methods. Jour. Path. and Bact. **28**: 203–209. 1925.

19. SALMON, E. S., and W. M. WARE. Department of Mycology. Jour. South-Eastern Agr. Col., Wye, Kent. **31**: 13–21. 1933. (Abstr. in Rev. Appl. Mycol. **12**: 353. 1933.)
20. SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIOLOGICAL TECHNIC. Manual of Methods for Pure Culture Study of Bacteria. Leaflet 2, Preparation of media. 9th ed., 1944. Leaflet 4, Staining methods. 9th ed., 1946. Leaflet 5, Routine tests for the descriptive chart. Morphological and biochemical. 11th ed., 1949. Leaflet 6, Further biochemical methods. 9th ed., 1942. Biotech. Pub., Geneva, N. Y.
21. STAPP, C. Eine bakterielle Faule an Lactuca sativa var. capitata L. and Cichorium endivia L. Centralbl. Bakt., II, **91**: 232–243. 1935.
22. SULLIVAN, M. X. Synthetic culture media and the biochemistry of bacterial pigments. Jour. Med. Res. **9**: 109–160. 1905.
23. SWINGLE, D. B. Center rot of "French endive" or wilt of chicory (Cichorium intybus L.). (Abstr.) Phytopath. **15**: 730. 1925.
24. THORNBERRY, H. H., and H. W. ANDERSON. Some bacterial diseases of plants in Illinois. Phytopath. **27**: 946–949. 1937.
25. WRIGHT, R. C. The freezing temperatures of some fruits, vegetables, and florists' stocks. U. S. Dept. Agr. Cir. 447. 1937.

LARVAL EMIGRATION FROM CYSTS OF THE GOLDEN NEMATODE OF POTATOES, HETERODERA ROSTOCHIENSIS WOLLENWEBER[1]

B. F. LOWNSBERY[2]

(Accepted for publication May 28, 1951)

O'Brien and Prentice (7) demonstrated that a diffusate from potato roots stimulates golden nematode larvae to emigrate from their cysts. This response has been widely used as a criterion of the viability of encysted larvae of this species. Smedley (12) added leachings from potted potato plants to equal numbers of cysts which had been treated with isothiocyanates. The relative lethal action of the treatments was determined by counting all the larvae emigrating from each lot. Gemmell (5) tested response to leachings by placing cysts in drops of the stimulant, one cyst in each drop. Both the counted-lot and the single-cyst method are tedious if sufficient cysts are used for an adequate representation of the total population, and both usually involve a selection of cysts, so that it is difficult to obtain a representative sample. The method presented herein has been useful for studying factors affecting emigration and determining viability of golden nematode larvae. Large numbers of cysts are used in each replicate and no selection of cysts is involved. It is thought that a similar assay might be useful in research with any species of *Heterodera* in which the bulk of the larval inoculum is encysted and the species responds to an excretion of the host. Removal of golden nematode larvae from their cysts in the field would seem to be advantageous for control purposes and this possibility is considered in this paper.

THE WEIGHED CYST LOT METHOD

A mixture of cysts and accompanying organic debris (Fig. 1) was obtained from the soil by means of agitation in the extraction tank (Fig. 2). Infested Long Island loam was put into the tank in 75-lb. lots. Sufficient agitation was provided by a 2-in. water line from the main to the tank, with 38 lb. pressure per sq. in., the water entering the tank through four nozzles at the base. When the water level was near the top of the tank the main valve was closed for a minute to allow separation of gravel from the floating material. The valve was then opened slightly and the floating material allowed to run over the spout onto a nested series of screens—4, 25, and 60 meshes to the inch. When all of the floating material was on the screens

[1] Excerpts from thesis presented June 1950 to the faculty of the Graduate School of Cornell University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Assistant Professor, Department of Plant Pathology, Cornell University, and Associate Nematologist, Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture.

The writer expresses his appreciation to Dr. W. F. Mai, Dr. A. G. Newhall, and Dr. Bert Lear for advice and assistance during the course of these studies.

the stopper was pulled out of the 4-in.-diameter drain in the bottom of the tank, and the contents dropped rapidly into a cesspool over which the tank was used. A stream of water was directed forcefully onto the nested screens

FIG. 1. Typical cyst mixture used in the emigration assay.

and then the cyst mixture was obtained from the 60-mesh screen. The mixture was dried, resieved dry, and thoroughly mixed. One-tenth-gram samples of this product were obtained by weighing with a torsion balance sensitive to 2 mgm. The mean cyst count in 30 samples of typical cyst mixture was 661, the standard deviation 42, and the coefficient of variability 6.

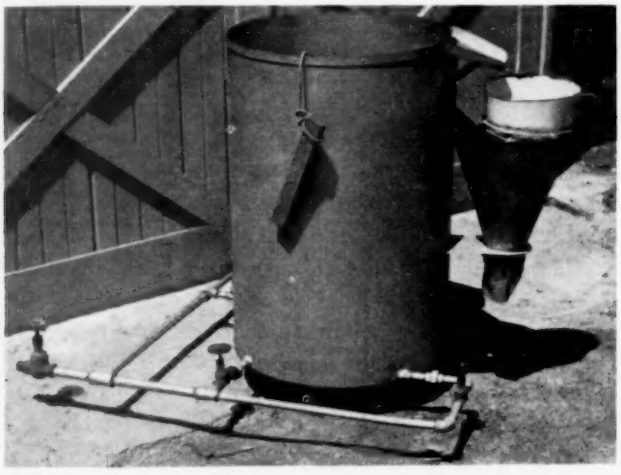

FIG. 2. Tank used to extract golden nematode cysts from the soil.

Treatments being tested for effect on larval emigration or viability were given to these weighed samples.

Each sample was then placed in a Petri dish with 25 ml. of leachings from a potted potato plant and incubated at 21° C. for 2 weeks. This temperature and time interval were favorable for a high rate of emigration (Tables 1 and 2).

TABLE 1.—*Emigration from golden nematode cysts into leachings from potted potato plants at eleven temperatures*

Temperature (degrees C.)	Emigrated larvae per 10-ml. sample		
	Mean of 5 replicates, Oct., 1948	Mean of 9 replicates, March, 1949	Mean of 8 replicates, Aug., 1949
3	0	0	0
6	0	0	0
9	0	0	0
12	0	0	0
15	5	0	426
18	31	3	585
21	30	17	704
24	45	8	683
27	51	6	576
30	0	2	142
33	0	0	0
L.S.D.: 5 per cent level	15	5	126
1 per cent level	20	7	167

TABLE 2.—*Number of larvae stimulated to emigrate from golden nematode cysts after soaking in leachings from potted potato plants for 18 periods of time, October, 1949*

Time (days)	Number of larvae[a]	Time (days)	Number of larvae[a]	Time (days)	Number of larvae[a]
1	0	7	38	13	328
2	0	8	87	14	362
3	0	9	122	18	456
4	0	10	161	22	592
5	3	11	217	30	614
6	12	12	312	34	481

L.S.D.: 5 per cent level, 129
1 per cent level, 171

[a] Mean of 5 replicates of emigrated larvae per 10-ml. sample.

After incubation, the contents of each Petri dish were washed into a 300-ml. beaker marked at the 100-ml. level, and the volume was brought to 100 ml. with water. A 10-ml. sample was pipetted from the beaker to a Syracuse dish while the beaker contents were stirred with an electric stirrer. Larvae in the Syracuse dish were counted using a stereoscopic microscope and a magnification of 12 times. Parallel lines $\frac{1}{4}$ in. apart, scratched in the Syracuse dish, aided in counting. A coefficient of variability of 8 was obtained for ten larval counts made from the same larval suspension in this manner. No less variability was shown when two 5-ml.

or four 2½-ml. samples were employed. Results of 12 weighed cyst lot emigration assays (Table 3) indicated that variation between replicates decreased as larval emigration increased.

TABLE 3.—*Variation in larval counts obtained using the weighed cyst lot emigration assay*

Number of larvae[a]	Standard deviation	Coefficient of variability	Number of larvae[a]	Standard deviation	Coefficient of variability
37	16	43	41	9	22
149	60	40	251	51	20
158	55	35	104	19	18
145	47	33	880	114	13
472	118	25	565	68	12
79	18	23	890	37	4

Coefficient of correlation:

Between means and coefficients of variability	−0.783
Necessary for significance at the 5 per cent level	−0.553
Necessary for significance at the 1 per cent level	−0.684

[a] Mean of 10 replicates of emigrated larvae per 10-ml. sample.

When only an aliquot fraction of the emigrated larvae are counted, very small numbers of emigrated larvae will be missed. With the above method an emigration of fewer than ten larvae per 0.1-gm. sample would not be detected. To detect such slight emigration it would be necessary to increase the size of the aliquot counted.

Use of air-dried cysts delays emigration, but drying is necessary for accurate weighing. The weighed lots might be soaked in water previous to the experiment to increase emigration rate. If this were done it would be necessary to eliminate larvae emigrating into the water by careful washing of the soaked cysts over a screen.

As outlined here, the weighed cyst lot method measures only total emigration after one period of time and exposure to one collection of leachings. When more detailed emigration information is desired, a portion of the method of Fenwick (3) might be incorporated.

Collection of potato-root diffusate for use in the weighed cyst lot method. The leachings from potted potato plants used with this assay were collected by placing the potted plant in a funnel on filter paper, watering the plant, and collecting the water as it leached through the soil and filter paper and dripped out the bottom of the funnel. To determine the extent to which a potted potato plant can be leached in this manner to obtain effective stimulant, water was added continuously to a potato plant growing in a 6-in. pot and forty 100-ml. volumes of this water were collected successively; every fifth 100-ml. collection up to and including the fortieth was saved. The filter paper was not changed between collections. Not more than 8 hr. were required for 40 l. of water to leach through the pot. The stimulating efficacy of each saved collection was tested, using the weighed cyst lot method. The experiment was repeated twice and the data from the three

experiments were combined for analysis of variance. The decrease in stimulation with continued leaching in the manner described here was gradual and there was significant stimulation by even the fortieth 100 ml. collected.

No differences were detected between stimulating efficacies of leachings from plants of different ages obtained by planting in the same thoroughly mixed soil at 2-week intervals, if differences in root weight were considered. Plants used ranged from 2 weeks old with very small root systems to 14 weeks old with roots showing some necrosis. Odds of greater than 19 : 1 but less than 99 : 1 were obtained for a correlation between root weight and stimulating efficacy of leachings from these same plants.

The stimulating efficacy of leachings was preserved for a year by freezing. Their efficacy was not destroyed by passage through a Seitz bacterial filter.

Effect of season on larval emigration and the weighed cyst lot method. As has been reported by Triffitt (13) and others, there is a seasonal variation in larval emigration. Failure of larvae to migrate from cysts in quantity in late fall, winter, and early spring limits use of emigration in viability assays to the remaining months of the year. Ellenby (2) speculated that this dormancy might be caused by an accumulation of metabolic products in the cyst, but Reinmuth (9) observed the same effect of season on the agility of the free larvae in water.

This seasonal dormancy was observed in New York State, and an experiment was made to measure it quantitatively. A hole, 1 yd. square and 8 in. deep, dug in the field, was filled with heavily infested soil which had been sifted through ¼-in.-mesh screen and thoroughly mixed. On July 20, 1949, cyst mixture was extracted from 25 lb. of this soil. The mean number of cysts in five 1/10-gm. samples of the cyst mixture was determined. A weighed cyst lot emigration assay was conducted July 31 to August 14 using this cyst mixture. Frozen leachings from potted potato plants, thawed immediately before use, and fresh leachings from potted tomato plants grown that summer were used as stimulants. A tap water control was included. Another portion of the cyst mixture extracted July 20 was stored dry in a constant-temperature chamber at 25° C. until December 16. The weighed cyst lot emigration assay was repeated December 16 to December 30, 1949. Another sample of the same frozen leachings was thawed and used as a stimulant along with fresh leachings from potted tomato plants grown in late fall. A tap water control was again included. The cyst mixture extracted in July and stored at 25° C. was used in this experiment together with cyst mixture extracted from the same soil on November 26, 1949 (after the soil had been exposed to freezing temperatures). The mean number of cysts in five 1/10-gm. samples was also determined for the latter cyst mixture. The low rate of winter emigration into the frozen leachings and tap water as well as into the fresh leachings from tomato plants (Table 4) indicates that dormancy is a part of the nematode physiology and is not caused by a winter difference in the leachings.

TABLE 4.—*Emigration of golden nematode larvae in August and December*[a]

Emigration	In August 1949 from cysts extracted July 1949	In December 1949 from cysts extracted	
		July 1949	November 1949
Into fresh leachings from tomato plants	117 ± 16	1 ± 2	1 ± 1
Into leachings from potato plants preserved by freezing	135 ± 23	6 ± 6	4 ± 2
Into tap water	34 ± 4	1 ± 1	1 ± 1

[a] Figures in the body of the table are means together with their standard deviations. They represent emigrated larvae per 10-ml. sample per 100 cysts.

In agreement with Reinmuth (9), it has been observed that exposure to winter conditions is not necessary for induction of this dormancy. For example, encysted larvae washed from the soil in summer became dormant in the winter even though they were kept at a summer temperature (Table 4).

Attempts to break this dormancy by centrifugation, reduction of atmospheric oxygen, and 23 different treatments involving temperature fluctuations combined with moistening and drying, were unsuccessful. None of these treatments, however, lasted longer than 1 week.

The many failures to modify the seasonal variation in larval emigration rate by temperature treatments lead the author to believe that temperature is not the factor directly governing the seasonal behavior.

EFFECT OF DEGREE OF MATURITY OF CYSTS ON LARVAL EMIGRATION

While larvae did migrate from current-year cysts before exposure to winter conditions (in agreement with Triffitt (14) and contrary to Reinmuth (9)), emigration was greater from cysts which were not taken from the roots until the spring following their formation. This increase might have been caused by the winter exposure which they received, but it might also have been caused by their longer stay on potato roots. Development of larvae to the point where they would emigrate from newly formed cysts required between 8 and 12 weeks from the time of larval infestation of the soil in which potato plants were growing. The mean soil temperature at a depth of 6 in. averaged 72° F.

STIMULATION OF EMIGRATION AS A FIELD CONTROL MEASURE

Rensch (10, 11) and Nebel (6) attempted unsuccessfully to find a practical control of the sugar-beet nematode, *Heterodera schachtii* Schmidt, by adding aniline and citric acid to the soil to stimulate emigration. Calam, Raistrick, and Todd (1) mention stimulation of larvae of *Heterodera rostochiensis* from their protecting cysts as a possible control. Rademacher and Schmidt (8) have pointed out some of the disadvantages of the method. They state that under field conditions a complete emptying of the cysts by stimulation cannot be obtained even by repeated treatment. Distribution

of the stimulant throughout the soil is a major problem. Triffitt (13) found that the natural stimulant is rapidly broken down under nonsterile conditions. It would not be expected to be effective long, therefore, in the field. To obtain actual data on the effect of adding golden nematode stimulant to the soil, the following two experiments were performed.

Each of six 10-in. pots was filled with 8 lb. of infested soil which had been sieved and thoroughly mixed. Two of these pots were used as controls. Leachings from potted potato plants were added to the other four once a week from July 19 to September 3. Two pots received 2 l. of leachings per week, a total of 14 l. or approximately 760,000 gal. per acre. The two remaining pots received 5 l. of leachings per week, a total of 35 l. or approximately 1,900,000 gal. per acre. All pots were stored in an unheated barn over winter. The following July the contents of each pot were screened and made uniform. Cysts and accompanying debris were floated and sieved from a 2-lb. sample of the soil from each pot. Twenty-five ml. of leachings from a potted plant were added to the cyst mixture representing each pot in order to compare the remaining larval potential in the cysts from the treated soil with that in cysts from the untreated soil. Emigration from cysts from treated and untreated soil was assayed by the weighed cyst lot method described above. A 94 per cent reduction in larval emigration potential was obtained by addition of 35 l. of leachings from a potted potato plant. The 14-l. treatment caused an 89 per cent reduction. The difference between the two reductions is not significant. In a similar experiment performed a year earlier a $3\frac{1}{2}$-l. addition (approximately 190,000 gal. per acre) had produced no reduction in larval emigration potential. Fenwick (4), in a similar experiment assayed in a different manner, found that soil treatment with leachings caused 84 per cent of the larvae to migrate from the cysts. Judging from these tests, a synthetic stimulant would be worthy of investigation for control if it were effective after tremendous dilution and if a practical method of adding the stimulant to the soil were found. Treatment with such a compound might shorten the rotation necessary between profitable potato crops.

SUMMARY

A method for assaying larval emigration from golden nematode cysts is described which uses large numbers of cysts and eliminates selection and counting of cysts. Weighed cyst lots are employed. Only a fraction of the larvae emigrating from each lot into leachings from potted potato plants during 2 weeks at 21° C. is counted. Variance in results obtained using this method increases as larval emigration decreases. Stimulating efficacy of leachings from potted potato plants decreases gradually as leaching continues and several ml. can be collected from a single potato plant at one time with little reduction in stimulating efficacy. Potency of the leachings increases with size of root system, but a correlation with age of plant was not detected. Because of a seasonal dormancy, use of the assay is lim-

ited to late spring, summer, and early fall. Attempts to break or delay this dormancy were unsuccessful. Emigration from current-year cysts into leachings occurred before these cysts were exposed to winter conditions.

In a pot experiment a 94 per cent reduction in larval emigration potential was obtained by addition of a large volume of leachings from potted potato plants to infested soil. It is concluded that a synthetic stimulant would be worthy of investigation for control if it were effective in soil at a tremendous dilution.

DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. CALAM, C. T., R. H. RAISTRICK, and A. R. TODD. The potato eelworm hatching factor. 1. The preparation of concentrates of the hatching factor and a method of bioassay. Biochem. Jour. **45**: 513–519. 1949.
2. ELLENBY, C. Ecology of the eelworm cyst. Nature **157**: 451–452. 1946.
3. FENWICK, D. W. Investigations on the emergence of larvae from cysts of the potato-root eelworm Heterodera rostochiensis. 2. The form of the hatching curve. Jour. Helminthol. **24**: 75–86. 1950.
4. ————. Investigations on the emergence of larvae from cysts of the potato-root eelworm Heterodera rostochiensis. 3. Larval emergence in soil under the influence of potato-root diffusate. Jour. Helminthol. **24**: 86–90. 1950.
5. GEMMELL, A. R. Studies in the biology and control of Heterodera schachtii Schmidt. West of Scotland Agr. Bul. 139. 1940.
6. NEBEL, B. Ein Beitrag zur Physiologie der Rübennematoden vom Standpunkt der Bekämpfung. Kuhn's Archiv **12**: 38–103. 1926.
7. O'BRIEN, D. G., and E. G. PRENTICE. An eelworm disease of potatoes caused by Heterodera schachtii Schmidt. Scottish Jour. Agr. **13**: 415–432. 1930.
8. RADEMACHER, B., and O. SCHMIDT. Die bisherigen Ehrfahrung in der Bekämpfung des Rübennematoden (Heterodera schachtii Schmidt). Archiv Pflanzenbau **10**: 238–296. 1933.
9. REINMUTH, E. Der Kartoffelnematode (Heterodera schachtii Schmidt). Beiträge zur Biologie und Bekämpfung. Ztschr. Pflanzenkrankh. **39**: 241–276. 1929.
10. RENSCH, B. Zur Frage der Nematoden Bekämpfung. Zuckerrübenbau (Neue Folge) **7**: 24–32. 1925.
11. ————. Zwei quantitative reizphysiologische Untersuchungsmethoden fur Rübennematoden. Ztschr. Wiss. Zool. **123**: 488–497. 1925.
12. SMEDLEY, E. M. Experiments on the use of isothiocyanates in the control of the potato strain of Heterodera schachtii Schmidt. Jour. Helminthol. **17**: 31–38. 1939.
13. TRIFFITT, MARJORIE J. On the bionomics of Heterodera schachtii on potatoes with special reference to the influence of mustard on the escape of larvae from the cysts. Jour. Helminthol. **8**: 19–48. 1930.
14. ————. Observations on the life cycle of Heterodera schachtii Schmidt. Jour. Helminthol. **8**: 185–196. 1930.

STUDIES ON A COLOR TEST FOR STONE FRUIT VIRUS DISEASES[1]

R. C. Lindner, T. E. Weeks, and H. C. Kirkpatrick[2]

(Accepted for publication May 29, 1951)

Colorimetric methods for detecting virus diseases in stone fruit trees, based upon the accumulation of polyphenols in diseased tissue, have been described (3, 5). Approximately 70,000 tests have been made to determine the most suitable plant tissues to sample, to establish color indices for different *Prunus* species, and to determine the usefulness and limitations of the method. This paper presents results of the numerous tests on sweet cherries, mahaleb cherries, and peaches, and the more limited tests on mazzard cherries, plums, prunes, and apricots. The potential significance of a color test for stone fruit virus diseases is greatest with regard to the mahaleb and mazzard varieties used as understocks for sweet and sour cherry varieties, and peach seedlings, used as understocks for peach varieties. In the past too little attention has been given to the possible presence of virus in understocks.

LEAF TESTS

The procedure for leaf samples, which has been described elsewhere (3), has consistently given very good results. The sample, a $\frac{1}{4}$-in. disk of leaf tissue obtained with a paper punch, is placed in a $\frac{1}{2}$-in. test tube and 5 ml. of reagent added. (The reagent is composed of 40 gm. sodium hydroxide, 0.3 gm. cupric sulfate, 3 gm. sodium citrate, and 1000 ml. distilled water.) The tube containing the sample and reagent is then heated in a boiling-water bath for 5–10 min., allowed to cool for about 10 min., and then shaken thoroughly before a reading is taken in the colorimeter. An alternative (heating) procedure consists of heating the test tube containing the sample and the reagent in a water bath at 80° C. for 20 min., shaking the tubes after 10 minutes' heating to allow for uniform extraction, and reading the samples in a colorimeter immediately after heating. The colorimeter reading remains constant for at least 30 min. after maximum color formation with either heating procedure. A Klett-Summerson photoelectric colorimeter (with green filter No. 54) has been used.

Precautions in sampling are very important if uniform results are to be secured. For tests on cherry trees, only the "adult" type of leaf such as that on the spurs or at the base of terminal growths should be used. On

[1] Scientific Paper No. 1001, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

[2] Respectively, Associate Plant Pathologist, Tree Fruit Experiment Station, Wenatchee, Washington; Technical Assistant in Plant Pathology, Washington State Department of Agriculture, Wenatchee, Washington; and Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Wenatchee, Washington.

The writers wish to acknowledge the cooperation of Earle C. Blodgett and E. L. Reeves in supplying plant samples affected with various virus diseases.

young cherry trees few leaves of this type are available except at the base of terminal growths. Samples should not be taken from girdled trees or branches. Certain types of winter injury, heavy San Jose scale infestations, and Coryneum blight infections interfere with the test. Red spots on peach leaves resulting from such disorders as nitrogen deficiency or arsenic injury should likewise be avoided in sampling, but green areas on such leaves may be used.

Two areas are representative of the leaf as a whole. One area, the lamina sample, lies in the center of either half of the leaf, midway between the midrib and the leaf margin. The other area, the midrib sample, lies exactly in the center of the leaf. Table 1 indicates the variability to be

TABLE 1.—*Variability in colorimeter readings on samples from individual Bing cherry spur leaves showing symptoms typical of the disease in question, in September*

Disease	Sample position (apical to basal)[b]	Colorimeter readings[a]		
		Left lamina	Right lamina	Midrib
Mild form of latent complex[c]	1	28	28	43
	2	29	36	
	3	36	38	41
	4	31	35	
	5	41	41	56
Severe mottle-leaf complex	1	268	212	124
	2	288	132	
	3	194	127	121
	4	141	110	
	5	197	67	138
Mild rusty-mottle complex	1	325	185	166
	2	100	375	
	3	250	262	244
	4	155	240	
	5	145	240	222
Severe rusty-mottle complex	1	515	264	520
	2	272	290	
	3	405	320	420
	4	345	254	
	5	345	280	410
Western X–little-cherry complex	1	520	400	410
	2	540	430	
	3	460	317	375
	4	375	264	
	5	214	206	218

[a] Readings indicate intensity of red color. The higher the reading, the more intense is the color.

[b] 1 indicates the apical position on the leaf; 5, basal position.

[c] The term "virus complex" is used to indicate the presence of more than one virus. The term "latent complex" is used to denote an unknown number of viruses, which produce meager or no obvious symptoms (6). Such complexes are almost universally present in cherry trees in the Pacific Northwest.

expected in testing any individual spur leaf for any one of several cherry diseases. The variability for peach leaves has been much the same as that for cherry leaves.

The data in table 1 are representative of diseased leaves whose symptom expression is typical for the disease in question. During initial stages of infection in a leaf, however, somewhat different results may be expected. Usually the apical portion of the leaf is the first to give a chemical test. Frequently the first effects have been more on one side than the other.

For diagnostic purposes a number of carefully chosen samples from each tree must be tested, since there is variability in samples from the same leaf as well as in samples from different leaves. We have been using a minimum of five leaf samples per tree and averaging the results.

Seasonal variation was tested in leaf samples obtained from the foliage of certain diseased trees at intervals throughout the growing season. Figure 1 shows the results of midrib tests for three virus complexes.

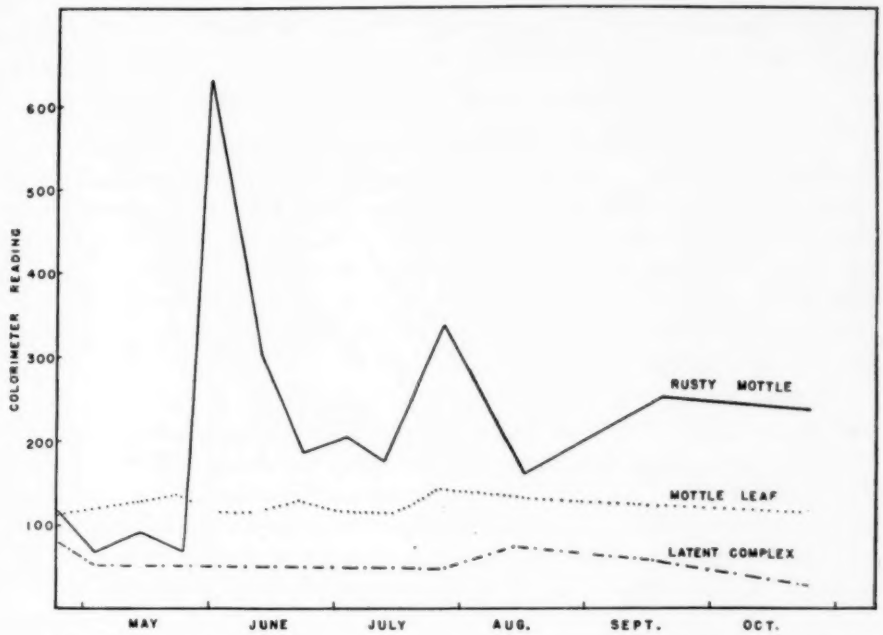

FIG. 1. Seasonal variation in colorimetric readings on midribs of Bing cherry leaves affected with three virus complexes.

The readings for five leaf samples collected at random from each diseased tree without regard to symptom expression were averaged. No attempt was made to use only leaves with comparable symptoms. The averages apparently indicate different virus concentrations in the foliage at different times of the growing season. When only those leaves that show approximately equal symptom expression of rusty mottle are tested at different periods of the growing season, relatively little variation is found in the readings. Readings for the mottle-leaf and latent complexes fluctuate very little during the season. Rusty-mottle complex readings reach a very high primary peak approximately 1 month following full bloom, about the time

when maximum symptom expression occurs in the foliage. One or two secondary peaks were recorded later in the season. It appears that the rusty-mottle virus either is not present in the foliage or is at a very low concentration early in the season, but that it rapidly builds up to a maximum about 30 days following full bloom. The low readings after this peak coincide with the loss of badly affected leaves from the tree; the leaves remaining on the tree have a low virus content.

Western-X disease of peaches and the Western-X–little-cherry disease of sweet cherries give readings somewhat similar to those of rusty mottle in that early in the season the foliage apparently contains a very low concentration of the virus. They differ from rusty mottle in that their occurrence is usually much more erratic in various parts of the tree during the rest of the growing season.

Leaf petioles are unsatisfactory for testing. Not only is it difficult to secure uniform samples from them, but the nectaries on the petioles contain compounds that produce interfering colors.

TESTS ON FLOWERS, FRUITS, AND SEEDS

Flowers selected from cherry and peach trees affected with various virus diseases were dissected and the pedicels, calyxes, petals, stamens, and pistils were tested colorimetrically. None of these tissues was satisfactory for a test for routine use. However, petals from cherry trees affected with the mottle-leaf complex consistently gave comparatively high colorimeter readings, as did an occasional pistil from cherry trees affected with the Western-X–little-cherry complex.

In most cases fruit tissue is not satisfactory because the high concentration of sugar that it contains precipitates cuprous oxide from the reagent. A modified procedure was developed for cherry fruit tissue and proved satisfactory for the Western-X–little-cherry disease (5).

Preliminary studies on stone fruit seeds indicate that the color test may be of value in determining the seed transmission of certain viruses.

WOOD AND BARK TESTS

The wood tissue of stems was suitable for routine tests, but that of roots gave variable readings. Bark tissue of either tops or roots is unsatisfactory, because it contains too many materials that produce interfering colors.

The method used for wood samples is essentially the same as that used for leaves, except that the heating is extended to 30 min. in a water bath at 80° C. The test tubes containing the sample should be shaken thoroughly several times during the heating to allow uniform extraction from the sample.

Wood samples have been obtained from current-season terminal growth. The internodal area in the mid-portion of the growth is used. The bark and all tissue adhering to the wood are scraped off with a sharp knife. A 5- to 10-mgm. sample of the scraped wood, dried at 80° C. in a vacuum oven overnight, is weighed and tested as indicated above. A blue filter

(Klett No. 42) may be used as a more sensitive measure of the red color instead of the green filter previously described. The final colorimeter reading is converted to read per 10 mgm. (dry-weight basis). Wherever possible, twigs of similar diameter should be used to insure samples of a relatively uniform tissue composition. It is desirable to take at least five different samples per tree and to average the results to reduce the variability error for a given tree.

Wood tests are more difficult to make and more time-consuming than leaf tests, but they are of value during the dormant season when foliage is not available. Furthermore, wood tissue of peach and mahaleb trees is more satisfactory than foliage when the test is for the latent complex. A summary of the results of tests on peach wood is in table 2. Arbitrary

TABLE 2.—*Ranges in colorimeter reading of wood samples of peach trees affected with various virus diseases*

Arbitrary class	Colorimeter reading per 10 mgm. of dry wood		Disease complex
	Green filter	Blue filter	
0	20– 40	60– 80	Probably virus-free
1	40– 55	80–130	Mild latent complex
2	55– 70	130–180	do
3	70– 85	180–230	Moderate latent complex
4	85–110	230–310	Severe latent complex
5	110–140	310–400	Western X-disease complex
6	140 +	400 +	do

classes have been set up within certain color ranges, and the virus diseases that fall within each class are indicated.

Our results indicate that such virus diseases of peaches as wart, mosaic, and several undescribed diseases are not distinguishable from the latent complex by the wood test. Wood samples from trees affected with these diseases test within the various latent classes. The staining test on the foliage (4), however, is useful in distinguishing between some of these diseases.

A summary of the results of tests on mahaleb wood is in table 3. For

TABLE 3.—*Ranges in colorimeter readings for wood samples of mahaleb trees affected with various virus diseases*

Arbitrary class	Colorimeter reading per 10 mgm. of dry wood		Disease complex
	Green filter	Blue filter	
0	10– 25	20– 40	Probably virus-free
1	25– 40	40– 60	Mild latent complex
2	40– 60	60– 85	Moderate latent complex
3	60– 75	85–105	Severe latent complex
4	75– 95	105–150	Undescribed
5	95–120	150–200	Undescribed
6	120 +	200 +	Undescribed

mahaleb trees, as with peach trees, the wood test is more sensitive than the foliage test for the latent complex.

Transmission of the ring-spot virus through mazzard cherry seeds (2) and of the sour-cherry yellows virus complex through mahaleb cherry seeds (1) has been described previously, but less than 10 per cent of the seed were reported to be affected. Color tests in these studies indicate that certain portions of the latent complex are transmitted through more than 90 per cent of mazzard, mahaleb, and peach seeds.[3] Thus, the color test either (a) is a more sensitive index to the presence of ring spot and sour-cherry yellows, or (b) it indicates the presence of previously undescribed components of the latent complex that are transmitted through stone fruit seeds. Most of the understocks for cherry and peach trees, therefore, probably contain some form of the latent complex before they are top-worked to a given variety. Evidence is accumulating indicating that very few, if any, orchard cherry or peach trees are free of all viruses.

SUMMARY

A study was made, mainly of cherry and peach trees, to determine which tissues would be the most sensitive and best adapted for chemical testing for the presence of virus diseases.

Leaves appear to be the most suitable tissue for testing for virus diseases on cherry trees, but wood also gives satisfactory readings and may be used during the dormant season.

Wood is the best tissue to use when testing for the latent virus complex on peach or mahaleb trees. A procedure for testing wood tissue is described.

The chemical test is useful in indicating the presence of virus infection in stone fruit trees. Its chief limitation lies in the fact that it is not possible to determine exactly whch particular virus or viruses may be present.

The results from some 70,000 tests indicate that very few, if any, peach or cherry trees appear to be free of viruses. Portions of the latent complex appear to be transmitted through most, if not all mazzard, mahaleb, and peach seeds.

TREE FRUIT EXPERIMENT STATION
WENATCHEE, WASHINGTON

LITERATURE CITED

1. CATION, DONALD. Transmission of cherry yellows virus complex through seeds. Phytopath. **39**: 37–40. 1949.
2. COCHRAN, L. C. Passage of the ring spot virus through "mazzard cherry seeds." Science **104**: 269–270. 1946.
3. LINDNER, R. C. A rapid chemical test for some plant virus diseases. Science **107**: 17–19. 1948.
4. ————, H. C. KIRKPATRICK, and T. E. WEEKS. A simple staining technique for detecting virus diseases in some woody plants. Science **112**: 119–120. 1950.
5. ————, T. E. WEEKS, and H. C. KIRKPATRICK. A qualitative chemical test for some stone fruit virus diseases. Phytopath. **39**: 1059–1060. 1949.
6. MILBRATH, J. A., and S. M. ZELLER. Latent viruses in stone fruits. Science **101**: 114–115. 1945.

[3] Since this paper was written, the transmission of ring-spot virus through peach seed has been reported. Cochran, L. C. Passage of the ring-spot virus through peach seeds. (Abstr.) Phytopath. 40: 964. 1950.

A COMPARISON OF METHODS FOR EXTRACTING TOBACCO MOSAIC VIRUS FROM LEAF TISSUE

WILLIAM N. TAKAHASHI

(Accepted for publication May 28, 1951)

Only a small proportion of tobacco mosaic virus is extracted by macerating tissues in a food grinder and then pressing juice from the leaf macerate. The press cake, which is usually discarded, was shown by Bawden and Pirie (2, 3) to be the source of considerably more virus. These workers believe that the virus is bound in some manner to the cell residue and can be released only by disintegration of the residue by milling or by treatment with certain enzymes.

In view of this work by Bawden and Pirie it is now clear that in extracting virus from infected tissue the residue virus should not be disregarded. The efficiency of some current methods of extracting virus as well as the more elaborate ones of Bawden and Pirie where special effort has been made to account for the residue virus are compared in the following work.

Methods of extracting virus from plant tissue are of three types: 1) coarse maceration, 2) fine maceration or homogenization, and 3) coarse or fine maceration followed by enzyme digestion of the residue.

COARSE MACERATION

Well expanded leaves on previously topped plants of Turkish tobacco, *Nicotiana tabacum*, were inoculated with a stiff-bristled brush charged with a 1:1 dilution of crude juice from mosaic leaves. The excess inoculum was washed off. After 30 days of infection the inoculated leaves were harvested, weighed, and frozen. While still frozen, the leaves were passed through a fine-bladed food grinder. To the coarse macerate powdered K_2HPO_4 equivalent to 3 per cent of the original weight of the leaves was added. This raised the pH of the juice (5.6) to slightly above 7.0. Wringing the thawed macerate in cheesecloth extracted most of the juice; more was obtained with a Carver press until each 100 gm. of leaves yielded 78 ml. of juice. The virus was isolated by two alternate cycles of low- and high-speed centrifugations. This method is perhaps most widely used. The virus isolated in this manner is commonly called the juice virus.

This purified virus was assayed by determining the ultraviolet absorbancy (optical density) of a known dilution in a Beckman spectrophotometer at 265 mμ (5). From a preconstructed curve relating absorbancy (optical density) to mg. of virus nucleoprotein per ml., the value of the absorbancy was calculated as mg. of virus per gm. original fresh weight of tissue. This coarse maceration method yielded 3.75 mg. of virus per gm. of fresh tissue.

HOMOGENIZATION

Infected leaves were prepared as in the previous method. After harvesting, the midribs and the large lateral veins were cut out, the remaining tissue was minced into pieces measuring about 0.5 cm. square, and the pieces were shaken in a large beaker for randomization. One-gram lots were quickly weighed out, wrapped in aluminum foil, then placed in a tight jar and frozen.

The frozen 1-gm. samples were homogenized in a motor-driven ground-glass homogenizer (6). During this operation, 10 ml. of the appropriate buffer (0.1M phosphate pH 5, 6, or 7) were added gradually. The liquid was separated from the residue by centrifuging the slurry for 15 min. at 10,000 × gravity. The resulting supernatant liquid was ultracentrifuged for 1 hr. at 80,000 × gravity. The compacted pellet was then suspended in 2.5 to 5.0 ml. of 0.01M phosphate buffer of pH 7.0. Another cycle of low- and high-speed centrifugation was run to complete the purification of this fraction. In table 1 and in the discussion this virus has been designated as homogenate virus.

TABLE 1.—*Yield of tobacco mosaic virus (mg. per gm. fresh weight) from 30-day infected Turkish tobacco leaf tissue by the homogenization and enzyme-digestion methods*

pH	Homogenate method	Enzyme-digestion method			
		0° C.	Autolysis	Trypsin	Liver	Crop
5.0	Homogenate virus	6.85	6.65	6.85	7.10	6.10
	Residue virus 1	1.25	1.55	2.37	1.60	2.53
	Do 2	0.10	0.31	0.22	0.45	0.65
	Do 3	0.07	0.09	0.01	0.11	0.51
	Total	8.27	8.60	9.45	9.26	9.79
6.0	Homogenate virus	7.30	7.65	7.50	7.30	7.65
	Residue virus 1	1.25	1.15	1.59	1.35	1.35
	Do 2	trace	0.10	0.20	0.31	0.62
	Do 3	trace	trace	0.07	0.21	0.56
	Total	8.85	8.90	9.36	9.96	10.18
7.0	Homogenate virus	7.53	7.65	7.10	7.00	7.50
	Residue virus 1	1.29	1.22	1.44	1.23	1.20
	Do 2	0.05	0.05	0.16	0.42	0.63
	Do 3	trace	trace	0.08	0.31	0.51
	Total	8.87	8.92	8.76	8.96	9.84

The remaining green residue of each 1-gm. sample was mixed with 10 ml. of phosphate buffer and 3 drops of chloroform and stored at 0° C. for 8–12 hr. The mixture was homogenized again and the same procedure used to isolate the homogenate virus was repeated. This virus has been designated the residue virus; its yield is shown in table 1. The same residue was extracted a second and a third time in the same way (residue virus 2 and 3).

ENZYME DISINTEGRATION OF MACERATE

In this group the frozen 1-gm. samples were prepared up to the first residue in the same way as in the homogenization method. The virus of the supernatant liquid was isolated as described previously and is equivalent to the homogenate virus. To the residues 10 ml. of buffer were added. These suspensions were 1) autolysed and were digested with 2) commercial trypsin (Difco 1:250), 3) liver contents of snail, *Helix aspersa* L., and 4) crop contents of snail. Fresh snail liver was prepared by lyophilizing to dryness, then grinding the pieces to a fine powder in a Wiley mill. The crop contents were prepared according to Bawden and Pirie (3). Twenty mg. of trypsin or liver powder and 1 ml. of crop contents were used. During incubation (8–12 hr.) at 37° C., 3 drops of chloroform were added to each preparation to suppress the growth of microorganisms.

The virus of the incubated mixture was isolated in the usual way. The digest residue was again homogenized, treated with a fresh charge of enzyme, and incubated for a similar period. In all, three successive digestions were carried out. The virus portions extracted from these residues have also been called the residue viruses 1, 2, and 3. Yields are in table 1.

Before a complete experiment such as is shown in table 1 was attempted, numerous trials were run in separate sections but not always with the same lot of leaf tissue. Although the absolute values did not always agree, the trend of each experiment was similar to that shown.

COMPARISON OF VIRUS YIELDS

Coarse maceration of frozen tissue, as stated earlier, gave an average yield of 3.75 mg. of juice virus per gm. fresh weight of tissue. This is more than twice the average yield of juice virus from unfrozen coarse macerate (1). Freezing of tissue, then, is a prerequisite step in the extraction of virus.

The homogenization of frozen tissue with phosphate buffers at pH 5, 6, and 7 yielded an average of 6.71, 7.48, and 7.36 mg. of virus per gm. fresh weight. When these were added to the first residue virus the yield was increased to 7.96, 8.73, and 8.65, or almost 2.5 times that of the preceding method. Homogenization, pH control, and the extraction of the residue virus are important procedures in extracting virus from tissue.

In the enzyme method, digestion of the residue with trypsin, liver, or crop enzymes showed appreciable gain in virus. Autolysis did not increase the yield. In repeated trials including the experiment in table 1 the best yields with commercial trypsin, however small, were always at pH 5.0. On the other hand, the yields with liver or crop enzymes were highest at pH 6.0.

The combined yield of virus from the juice and from the residue incubated with snail crop enzyme obtained by Bawden and Pirie (3) amounted to 100 mg. of virus per gm. dry weight of leaves. If the dry weight of their leaves is taken as 10 per cent, then their yield converted

to wet weight would be around 10 mg. per gm. of fresh weight. The highest yield, 10.18 (Table 1), with use of a similar method of extraction, compares favorably with this. Bawden and Pirie (2, 3) found that only one-third of the virus was contained in the juice; the other two-thirds was liberated from the residue partly by milling and a great deal more by incubation with enzymes. In contrast to this, the present work shows that homogenization is more important than the use of enzymes in liberating virus; 87 per cent of the virus was extracted without the aid of enzymes. Similar observations were made by Limasset, Cornuet, and Gendron (4), who concluded that the enzyme merely disintegrates the cell and does not sever some special bond that holds the virus to the cell residue.

A rating of the efficiency of the various methods tested in this work can be made by taking the highest yield, 10.18, of the snail crop enzyme method as 100 per cent. The highest yields of the other methods compared with this are: coarse maceration, 37 per cent; homogenate, pH 7.0, 87 per cent; enzyme disintegration: autolysis, pH 7.0, 88 per cent; commercial trypsin digestion, pH 5.0, 93 per cent; and snail liver enzyme digestion, pH 6.0, 97 per cent.

Since the homogenate virus represents almost 75 per cent of the extractable virus, a single homogenization seems sufficient to prepare suitable inocula for most routine work. Where maximum yields are required there is, at this time, no recourse but to use the enzyme method, with successive incubations of the residue with crop contents of snail. Lacking snail enzymes, readily available commercial trypsin may be substituted with but 7 per cent loss.

SUMMARY

Three general methods of extracting tobacco mosaic virus from leaf tissue are described and compared: extraction of the virus from 1) coarse macerate of frozen tissue, 2) homogenate of frozen tissue, and 3) enzyme digest of the leaf residue.

The highest yield, 10.18 mg. per gm. fresh weight of tissue, was obtained by the enzyme digest method using crop enzymes of snail at pH 6.0. When this value is taken as 100 per cent the efficiency of the other methods are: coarse macerate method 37 per cent; homogenate method 87 per cent; and the enzyme digest method, commercial trypsin, pH 5.0, 93 per cent, and snail liver enzyme, pH 6.0, 97 per cent.

Homogenization is more effective in liberating virus than is enzyme digestion.

DIVISION OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BAWDEN, F. C. Plant viruses and virus diseases. 335 pp. Chronica Botanica Co. Waltham, Mass. 1950.
2. ————, and N. W. PIRIE. The liberation of virus, together with materials that inhibit its precipitation with antiserum, from the solid residues of tomato plants suffering from bushy stunt. British Jour. Exp. Path. **25**: 68–80. 1944.
3. ————, and ————. The virus content of plants suffering from tobacco mosaic. British Jour. Exp. Path. **27**: 81–90. 1946.
4. LIMASSET, P., P. CORNUET, and Y. GENDRON. Comparaison entre l'extraction du virus de la mosaique du tabac par voie mecanique et sous l'action des enzymes du tube digestif de l'escargot. Ann. Inst. Pasteur **78**: 135–138. 1950.
5. TAKAHASHI, WILLIAM N. Ultraviolet absorption as a measure of tobacco-mosaic virus nucleoprotein. Phytopath. **41**: 142–145. 1951.
6. ————. A ground glass homogenizer for preparing virus samples from plant tissue. Phytopath. **41**: 481–482. 1951.

RELATION OF PLANT SPECIES INOCULATED TO EFFICIENCY OF APHIDS IN THE TRANSMISSION OF BRASSICA NIGRA VIRUS

EDWARD S. SYLVESTER AND JOHN N. SIMONS[1]

(Accepted for publication June 1, 1951)

Severin[2] has presented evidence which would suggest that the host plant inoculated by an insect vector is of considerable importance in determining the percentage of infection obtained. For example, two races of the aster leafhopper, *Macrosteles divisus* (Uhler), have been reported to differ in their ability to infect celery, but not asters, with the aster-yellows virus. Similar results, reported in this paper, have been obtained during transmission experiments with the *Brassica nigra* virus and two vector species, viz., the green peach aphid, *Myzus persicae* (Sulzer), and the false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis).

MATERIALS AND METHODS

The *Brassica nigra* virus, described originally by Takahashi,[3] has been maintained in this laboratory in mustard, *Brassica juncea* Coss., and pak choi, *B. chinensis* L.

Both vectors used occur naturally upon cruciferous crops. Noninfective colonies of the green peach aphid were maintained on both sugar beets and pak choi, those of the false cabbage aphid upon stock plants, *Mathiola incana* R.Br. var. *annua* Voss, and pak choi. The insect transfers were made with a camel's-hair brush.

Two species of crucifer, mustard and pak choi, served as both virus source plants and test plants. The plants were started in flats, transplanted into 4-in. clay pots, and inoculated while in the cotyledon or early seedling stage. At the end of the aphid feedings, the plants were fumigated with nicotine and then placed in the greenhouse for incubation.

The following combinations of vector-virus source plant and virus test plant were used: the green peach aphid acquiring virus from infected pak choi, inoculating pak choi, and the same, except inoculating mustard; the green peach aphid acquiring virus from infected mustard, inoculating pak choi, and the same except inoculating mustard. The procedure was repeated using the false cabbage aphid as the vector. The infection feedings[4] were timed with a stop watch. The insects were starved (preinfection feed-

[1] Respectively, Assistant Professor of Entomology and Assistant Entomologist in the Experiment Station, and Research Assistant, Division of Entomology and Parasitology; University of California, Berkeley, California.

[2] Severin, Henry H. P. Newly discovered leafhopper vectors of California aster-yellows virus. Hilgardia 17: 511–519. 1947.

[3] Takahashi, William N. The morphology and host range of Brassica nigra virus. Amer. Jour. Bot. 36: 533–535. 1949.

[4] Watson, M. A. The transmission of beet mosaic and beet yellows viruses by aphides; a comparative study of a non-persistent virus and a persistent virus having host plant and vectors in common. Proc. Roy. Soc. London B, 133: 200–219. 1946.

ing starvation period) prior to the infection feeding upon the virus source plant.

Six replications, 10 plants per treatment, were made with each combination. In any particular replication, the sequence of use of the various insect-plant combinations was randomized.

RESULTS

The mean (range) values for the preinfection feeding starvation period, the infection feeding, and the test feeding period,[4] were as follows: the green peach aphid: 2.1 hr. (0.58–3.6 hr.), 15.7 sec. (13.8–20 sec.), and 2.3 hr. (0.91–4.0 hr.); for the false cabbage aphid: 1.9 hr. (0.38–2.9 hr.), 17.0 sec. (14.2–20 sec.), and 2.7 hr. (1.1–4.2 hr.).

TABLE 1.—*The relation of vector species, virus source species, and test plant species, to transmission of Brassica nigra virus*

Vector species	Virus source plant species	Test plant species	Number infected, of 60 inoculated[a]
Myzus persicae	Pak choi	Pak choi	0
		Mustard	23
	Mustard	Pak choi	1
		Mustard	27
Rhopalosiphum pseudobrassicae	Pak choi	Pak choi	8
		Mustard	11
	Mustard	Pak choi	3
		Mustard	7

[a] Six replications of 10 plants each were inoculated in each case.

The results (Table 1) show that the green peach aphid was a more efficient vector than the false cabbage aphid (51/240 infections to 29/239, $\chi^2 = 7$, approximately). The efficiency of the vectors was not dependent upon the species of plant which served as the virus source (42/239 transmissions from pak choi, 38/240 transmissions from mustard) and, of the two plant species inoculated, the mustard was the more susceptible (68/240 mustard infected to 12/239 pak choi infected).

The green peach aphid apparently had difficulty in inoculating pak choi plants (1/120 pak choi infected compared to 50/120 mustard infected), but this difficulty was not encountered to the same degree by the false cabbage aphid (11/119 pak choi infected compared to 18/120 mustard inoculated, $\chi^2 = 1.76$). In transmitting the virus to pak choi, the false cabbage aphid was a more efficient vector than the green peach aphid (11/119 transmissions compared to 1/120 transmissions, $\chi^2 = 7.6$). The reverse, however, was true when the test plant was mustard (18/120 transmissions to 50/120 transmissions, $\chi^2 = 20$, approximately).

It would appear that the vector efficiency in this particular combination of vector-host plant is dependent not only upon the species of insect used but also upon the species of host plant inoculated. Why the green peach aphid should lack the ability to readily inoculate one species of susceptible

host plant whereas the false cabbage aphid did not is a question which lacks an obvious explanation. Hypothetically, the results could indicate that an insect inoculation is dependent upon a specific compatibility between the virus, the insect salivary secretions, and the cellular contents of the inoculated plant cells. Another possible answer would be that the green peach aphid wandered off the pak choi, but not the mustard, whereas the false cabbage aphid was more equally stable, in regard to feeding, upon both hosts.

The green peach aphid is often reared in the noninfective state on pak choi at this laboratory, and no difficulty was encountered with this species during the infection feedings. To check the wandering tendency of the green peach aphid, individuals were starved, given a short infection feeding on a diseased plant, and then placed singly on each of 150 plants, 75 of which were pak choi and 75 mustard. After a test feeding of 30 to 60 min., the plants were checked to determine the presence or absence of the aphids. One aphid was missing of the 75 put on mustard; 6 were missing of those placed on pak choi ($\chi^2 = 3.7$). The tendency for more frequent wandering from the pak choi did not appear to be strong enough to account for the differences in transmission.

SUMMARY AND CONCLUSIONS

The results of trials designed to determine the efficiency of virus transmission by two vector species, that acquired virus from and inoculated virus into two plant species, indicated that the green peach aphid was able to inoculate the *Brassica nigra* virus into mustard plants with a fair degree of success, but was unable to effectively inoculate pak choi plants. The false cabbage aphid operated at a lower level of efficiency but had a less pronounced selectivity as to host plant species inoculated. Consequently the rating of the comparative efficiency with which vectors will transmit a virus could be misleading; e.g., the green peach aphid was a better vector if transmission was to mustard, but the false cabbage aphid was a better vector if transmission was to pak choi. The species of plant used as the virus source had but little effect upon vector efficiency.

Such interactions between a vector species and a host plant species must be considered when vectors are evaluated in relation to spread of a virus in the field.

DIVISION OF ENTOMOLOGY AND PARASITOLOGY,
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

THE EFFECT OF DYES IN RETARDING THE DEVELOPMENT OF CROWN GALLS

A. E. Dimond, E. M. Stoddard, and Saul Rich

(Accepted for publication June 4, 1951)

In 1941 Ark described the use of clove oil and of 3,5-dinitro-o-cresol as a chemotherapeutant for crown galls on almond (1). Evidently this work stimulated the interest of others, since Brown and Boyle (3, 4) reported 3 years later that penicillin, when applied to galls, caused them to become necrotic and eventually to disappear. In 1948 and 1949 a series of papers (2, 5, 6, 10) appeared which confirmed the results on penicillin and described a similar chemotherapeutic activity of streptomycin and of those folic acid derivatives which are also effective against cancer cells in animals (7).

Most recently, Nickell (12) has reported upon the effect of dyes on growth *in vitro* of virus tumor tissue from Rumex. Virus tumor tissue was grown in culture and dyes were added to the culture medium. Certain dyes, such as methylene blue, crystal violet, and malachite green, inhibited the growth of such tumors markedly but not permanently. Four dyes stimulated the growth of virus tumor, and it is of interest that all of them are specific stains for nucleic acids or nucleoproteins.

In all of these cases the action of the compound is upon the developed gall tissue. Determining whether galls can be retarded in their early development by compounds introduced into the plant was the objective of the work reported in this paper. In selecting compounds for these experiments, a second point was borne in mind. The compounds used by other authors are toxic to the pathogen *in vitro*. It is of interest to know whether nontoxic compounds are equally able to prevent gall formation. To this end the toxicity of a series of dyes was measured by incorporating the dye into sterile nutrient agar at a final strength of 0.0036 per cent, pouring it into Petri dishes, suspending bacteria in the melted but cooled agar, and counting the number of bacterial colonies arising after suitable incubation. Of the dyes listed in table 1, malachite green and brilliant green were highly toxic and prevented all colony formation in *Agrobacterium tumefaciens;* eosin B was moderately toxic; and the other dyes were either completely nontoxic or of very low toxicity at the concentration used.

Experiments on the effects of dyes on size of galls were carried out on Bonny Best tomatoes, grown in sand and fed with Hoagland's solution three times weekly. At the six-leaf stage they were treated with the dyes. Dyes were used at a concentration of 0.05 per cent[1] and were applied in one of two ways: (a) to the sand in which plants were growing, using 50 ml. per day for a 10-day period, or (b) by one injection with a hypodermic needle into

[1] After being distributed in the plant, the dyes would be present in a concentration which is roughly equivalent to that used in the toxicity tests noted above.

the vascular region of the stem just below the point of inoculation. After this treatment, plants were inoculated with cells from a 48-hr. culture of *Agrobacterium tumefaciens* (Riker's strain A-6) by stabbing a needle bearing inoculum into the stem. To avoid confounding, only one injection and one inoculation were made on each plant. Plants were then held for one month, at the end of which time galls were measured with a micrometer. Size of galls was estimated as the difference between diameter of gall plus stem and that of the stem alone.

Results of preliminary experiments indicated clearly that when dyes were applied to sand there was no reduction in gall size, but when hypodermically injected into the stem, certain dyes appreciably reduced the size of galls. Consequently a more elaborate experiment was set up in which dyes were applied by hypodermic injection only. In this experiment, three series of plants were treated, in which dyes were injected (a) 2 days before inoculation, (b) the same day, and (c) 1 day after inoculation. Each treatment was replicated five times. In table 1 are shown the effects of the various dyes upon the size of crown galls.

TABLE 1.—*The effect of dyes and the timing of treatment on the size of crown galls*

Dye	Size of galls (in millimeters)		
	Treated 2 days before inoculation	Treated on day of inoculation	Treated 1 day after inoculation
Methylene blue	3.2	4.5	5.9
Rhodamine B	3.8	5.0	6.4
Brilliant green	3.5	4.2	5.8
Malachite green	1.9[a]	5.8	5.6
Eosin B	2.8	5.6	5.5
Chlorphenol red	1.7[a]	6.2	6.3
Aniline blue	1.4[a]	6.0	
Check	4.8	6.6	6.6

[a] Reduction in size of gall is probably significant at odds of 20: 1 by analysis of variance.

Certain treatments, denoted in table 1, probably reduced the rate of gall development significantly. None of them prevented gall development, but it should be borne in mind that these effects resulted from but one injection of dye into the stem.

The time when treatment is made in relation to inoculation is critical if rate of gall development is to be significantly reduced. Evidently treatment must be made before inoculation. One may surmise that an interval of several hours must pass during which the dye moves to the site of inoculation. This interval must be longer than the time required for bacteria to become established in the host and start to grow, else injecting the stems the same day they are inoculated would also be effective. In this connection it was noted that the veins of leaves injected with certain dyes became col-

ored within a few minutes of the time when the plants were injected. In such cases the dye entered the vascular strand directly, but diffusion outward into the surrounding cells bearing inoculum almost certainly did not occur so rapidly.

One might postulate that the dyes, when injected into the host, were sufficiently toxic to affect the ability of host cells to divide. Such an effect would account for the reduction in gall size (Table 1). Presumably if an injected dye had such an effect, it would have to be of rather long duration to affect the rate of gall development significantly. Accordingly one would not expect the time when dyes were injected into the stem to be related critically to the time of inoculation. A related piece of research confirms this hypothesis. Dimond (8) has shown that when tomatoes are subjected to gamma radiation, host cells are inhibited in their division and this effect, at least in part, accounts for lack of gall development. In this case, timing is not at all critical, since inoculation may occur one week before irradiation begins or just after irradiation has ceased, but gall development is equally suppressed. In the present study, however, the relation of when plants were inoculated to when they were treated determined whether gall development was significantly retarded or not (Table 1). It is suggested, therefore, that reduction of gall development does not primarily result from a toxic effect of dyes upon the host cells which renders them unable to divide for a prolonged period of time.

Since experiments have here indicated that the dyes were not primarily effective because of their toxicity to the bacteria nor because they inhibited division of host cells, one may inquire how they were effective. Chemotherapeutants have been described already which are highly effective, but which are not toxic *in vitro* to the pathogen (9). Horsfall and Dimond (11) have suggested three probable modes of chemotherapeutic action: (a) upon the host itself, (b) upon the pathogen, and (c) upon the mechanism of pathogenesis, i.e., upon toxins liberated into the host. Any of these three mechanisms may be involved in the present case.

The lack of relation between toxicity of the dyes to bacteria *in vitro* and the ability of the dyes to reduce the rate of gall development are of interest. Aniline blue was nontoxic but gave greatest reduction in size of galls; chlorphenol red had similar properties. But malachite green, which was highly toxic, did reduce size of galls. Conversely, brilliant green, which is chemically very similar to malachite green, was toxic to bacteria but did not reduce gall development. There is no clear relationship between chemical structure and biological activity. The two most effective treatments are acid dyes, but not all acid dyes were effective. Malachite green, a basic dye, was similarly effective, whereas the closely related brilliant green was not.

In summary, one may say that gall size may be reduced by hypodermically injecting dyes into tomato stems just prior to but not after inoculating them with the crown-gall bacterium. The dyes which were effective

in this way were not always toxic to bacteria *in vitro*. There was no relation between chemical structure and either *in vitro* toxicity to bacteria or ability of the bacteria to reduce gall development.

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. ARK, P. A. Chemical eradication of crown gall on almond trees. Phytopath. **31**: 956–957. 1941.
2. BROWN, J. G. Cytological effects of penicillin and streptomycin on crown gall. (Abstr.) Phytopath. **38**: 3. 1948.
3. ————, and A. M. BOYLE. Penicillin treatment of crown gall. Science **100**: 528. 1944.
4. ————, and ————. Effect of penicillin on a plant pathogen. Phytopath. **34**: 760–761. 1944.
5. DEROPP, R. S. Action of streptomycin on plant tumours. Nature **162**: 459–460. 1948.
6. ————. The action of antibacterial substances on the growth of Phytomonas tumefaciens and of crown gall tumor tissues. Phytopath. **39**: 822–828. 1949.
7. ————. Inhibiting action of some analogues of folic acid on the growth of plant tumours. Nature **164**: 954. 1949.
8. DIMOND, A. E. Continuous gamma radiation suppresses crown-gall formation in tomatoes. (Abstr.) Phytopath. **41**: 10. 1951.
9. ————, and R. A. CHAPMAN. The chemotherapeutic properties of two compounds against Fusarium wilts. (Abstr.) Phytopath. **41**: 11. 1951.
10. HAMPTON, JACK E. Cure of crown gall with antibiotics. (Abstr.) Phytopath. **38**: 11. 1948.
11. HORSFALL, J. G., and A. E. DIMOND. The chemotherapy of plant diseases. Trans. N. Y. Acad. of Sci. 1951. (In press.)
12. NICKELL, L. G. Effect of certain dyes on the growth *in vitro* of virus tumor tissue from Rumex acetosa. Bot. Gaz. **112**: 290–293. 1951.

BACTERIAL FIRE BLIGHT OF RASPBERRY

MORTIMER P. STARR, CANUTO CARDONA[1], AND DONALD FOLSOM

(Accepted for publication May 29, 1951)

Early in August, 1947, a bacterial blight was observed on Latham red raspberry plants (*Rubus idaeus* L.) in a plantation in central Maine (5). The disease resembled fire blight of apple and pear and apparently was the disease that Lehman (7) described previously as occurring in North Carolina on raspberry. Since then the disease and the bacterial pathogen have been studied, with results reported here.

HISTORY

Detmers (3) in 1891 stated that a diseased raspberry cane found in Ohio was sent to T. J. Burrill, who replied by letter that the disease had been found in Illinois and was "pear blight." Heald (6, p. 325) published an illustration of a "poured-plate isolation showing colonies obtained from a bacterial blight of Antwerp raspberries." Although Elliott (4) lists the wild red raspberry, *Rubus strigosus*, and an unidentified *Rubus* sp. as hosts of *Erwinia amylovora*, we have not been able to discover the original report of this observation. Lehman (7) briefly described a bacterial twig and blossom blight of raspberry occurring in 1932 in North Carolina; found the bacterium pathogenic also on wild blackberry but not on apple; and pointed out that "apparently no one has published proof that raspberry is a suscept to *B. amylovorus*." The initial report on the raspberry blight in Maine (5) indicated the possible occurrence of raspberry blight in New Hampshire, and cited Lehman's unpublished observation of an outbreak in North Carolina during 1947.

THE DISEASE

Symptoms and signs. Infection of the young growing stem typically turns the stem purplish black. When the upper part of the shoot is infected, it dies and becomes black and curved. In 1947 and 1949, the disease broke out after blossoming time and there were some instances of infection in the inflorescences, which became purple and died. Later, inoculations in the greenhouse in midwinter showed that infection could be induced in the young inflorescence. The infection may start in the inflorescence and work down into the stem; it may start in a leaf and proceed into the stem and then up and down the stem from the node; or it may start in the stem and move into a leaf.

The leaf only may be infected; in fact several leaves on a shoot may be visibly affected with no stem lesions apparent. The leaf rachis becomes

[1] Fellow of the Rockefeller Foundation (1950–1951) at the Division of Bacteriology, University of California, Davis, California. On leave from the Facultad Nacional de Agronomia, Medellin, and the Ministerio Nacional de Agricultura y Ganaderia, Bogota, Colombia, S.A.

purplish black, the veins are blackened, and tissue adjoining the veins is brownish black (Fig. 1). Stem girdling at a node results in death of the leaf, which remains black and dried-out on the stem.

Sticky, milky drops full of bacteria ooze from the dark stem lesions, from green parts of the stem near the dark lesions, and from infected leaf veins. Blisters full of bacteria develop in the young bark.

Contributing factor. Rainy weather seems to be an important contributing factor as shown by the following circumstantial evidence. In 1947 and 1948, the plantation owner found the first infected specimen about

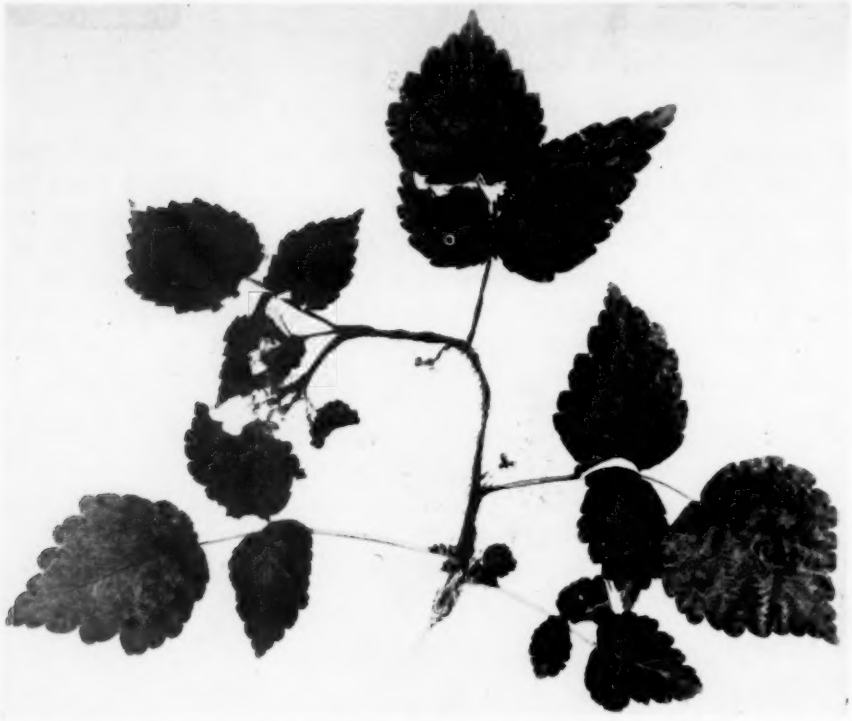

FIG. 1. Red raspberry infected with bacterial fire blight. Stem, leaf rachis, and veins blackened.

August 1. Before that date, apparently there was very little of the disease readily evident. In contrast to this, in 1949 a heavy rain occurred on July 10 and there was a sudden abundant appearance of the disease about 8 days after the rain. This is thought to indicate dispersal by rain. This belief is supported by greenhouse infections which can be induced by atomizing water suspensions of the pathogen on young raspberry shoots, and by the average incubation period of about 7 days.

Overwintering. In 1950 no infected shoots were found in the affected plantation on June 12 even though young canes of all sizes in the clumps of old and new canes were examined thoroughly. Further examination was not possible until July 19, when the disease was found in small late

shoots a few inches high, and in other shoots at heights of 9 to 18 in. Not until August 2 were symptoms found, in the upper parts of the most vigorous new canes. Possibly the pathogen overwintered in subterranean parts of the plants and was spread from the small late suckers to some of the inflorescences of the fruiting canes, and to the upper parts of the vigorous new canes before the owner began hoeing out the small suckers in the clumps of canes.

The disease was found, but only occasionally, in another plantation several miles away. Here the owner had planted in rows and had hoed up all suckers, an operation which would be easier and would therefore presumably be completed earlier, than in the clump system of cultivation. Wild raspberries growing in old pasture a few rods from the chronically affected Latham plantation were apparently free of the disease, and so cannot be considered in this instance as the overwintering source of infection.

Cross-inoculation attempts. In view of the similarity of the raspberry disease and apple fire blight, and of the causal agents of the two diseases, efforts were made to cross-infect apples and raspberry with the heterologous pathogens.

No infection resulted from stab inoculation with the raspberry pathogen into young McIntosh apple shoots and seedling trees forced in the greenhouse (1950–1951), although similar inoculations made at the same time with cultures of virulent *Erwinia amylovora,* originally isolated from apple, produced typical fire-blight symptoms. The *Erwinia amylovora* cultures did not infect raspberry shoots, although similar inoculations made at the time with the raspberry blight pathogen were effective.

ETIOLOGY—PROOF OF PATHOGENICITY

An attempt to isolate the pathogen in Maine in 1949 with dilution plates was unsuccessful, possibly due to the stickiness of the bacterial drops. However, a crude, possibly mixed, culture of the pathogen was isolated by direct transfer from fresh drops exuded from the stem through the green bark, and from inside the stem.

The pathogen was isolated in pure culture in California from diseased shoots sent from Maine in July 1949. Bits of infected tissue were teased apart in sterile water and plates were prepared using yeast-dextrose-$CaCO_3$ agar. Typical colonies were replated via suspensions in water to yield cultures of assured purity. Both Maine cultures and California cultures from Maine material produced infection in midwinter (1949–1950 and 1950–1951) in Maine in Latham red-raspberry shoots forced in the greenhouse. From these shoots the pathogen was reisolated in pure culture and used again to produce infection. From such infections, further pure cultures were obtained which in turn produced infection.

Stabbing small masses of bacteria into the young stem was always effective, provided these inoculated shoots were thereafter enclosed in wax paper. Infection resulted also when a water suspension of the bacteria

was atomized onto a young shoot in water-saturated air in an inoculation chamber.

The incubation period of the raspberry blight after artificial inoculation was usually 6 to 7 days, averaging 6.6 days for seven series of inoculations. A similar incubation period for natural infection can be surmised from the observation that 8 days after presumed inoculation during a heavy rain on July 10 there were abundant visible infections.

ETIOLOGY—DETERMINATIVE TESTS

Typical cultures of proven pathogenicity for raspberry were subjected to bacteriological determinative tests, the methods used being those which have been detailed elsewhere (8).

Morphology. Gram-negative, nonsporeforming, motile, rod-shaped bacteria with peritrichic arrangement of flagella.

Cultural reactions. Appearance of colonies and of broth cultures essentially identical with that given in the description of *Erwinia amylovora* in Bergey's Manual (1, p. 465).

Physiological characteristics. Indol not produced.

Not lipolytic by the spirit-blue agar method (10).

Gelatin liquefied.

Nitrite not produced from nitrate.

Acetylmethylcarbinol produced.

Methyl red test negative.

Litmus milk is not coagulated, but is slowly reduced; after 10–12 days it starts to turn alkaline, and thereafter is slowly peptonized. After 6 weeks, there is complete peptonization, and an alkaline reaction at the surface.

Acid without gas from glucose, maltose, and sucrose; no action on glycerin or lactose.

Utilizes citrate as shown by growth in Simmons citrate agar.

Utilizes naturally occurring asparagin as sole carbon and nitrogen sources (cf. reference 12 and reference 9, p. 254, for the technique used, and significance of this test).

Requires obligately the addition of nicotinic acid for growth in the synthetic medium (11).

ETIOLOGY—TAXONOMY AND NOMENCLATURE

The morphology of the raspberry pathogen, especially its arrangement of flagella, definitely places this microorganism in the genus *Erwinia*.

Solely from a bacteriological point of view, the cultural reactions, and particularly the obligatory nicotinic acid requirement (11), suggest that this is *Erwinia amylovora*.

However, we must consider the failure of apple strains of *E. amylovora* to infect raspberry, and the inability of the raspberry pathogen to infect apple. This suggests that the proper taxonomic procedure would be to indicate the bacteriological similarity and the phytopathological dissimilarity by naming the raspberry microbe as a special form of *E.*

amylovora. Such action would be in accordance with Recommendation 8a-5 of the proposed bacteriological code of nomenclature (2) which states that a "special form (*forma specialis*) is a subdivision of a species of a parasitic microorganism distinguished primarily by adaptation to a particular host." For this reason we recommend the designation *Erwinia amylovora* f. sp. **rubi**, f. sp. nov.

CONTROL

Varietal resistance is a possible means of control. For example, the disease has occurred for several years in rows of Lathams, but never has been found in a row of Newburghs alongside the Lathams. As suggested previously, growing in rows instead of in clumps and suppressing all new suckers not wanted for fruiting the next year might prevent epidemics.

SUMMARY

From an outbreak of a disease of Latham red raspberry in Maine, a bacterial pathogen was isolated in pure culture, proven to be the cause of this infection, and studied as to its determinative characteristics. The bacteriological traits of this pathogen suggest that it is closely related to the causal agent of apple fire blight; however, cross-inoculation tests between apple and raspberry using raspberry and apple pathogens show that the bacteria are different from a pathogenic viewpoint. The name *Erwinia amylovora* f. sp. *rubi* f. sp. nov. is suggested for this raspberry pathogen.

DIVISION OF BACTERIOLOGY
UNIVERSITY OF CALIFORNIA COLLEGE OF AGRICULTURE
DAVIS, CALIFORNIA, AND
AGRICULTURAL EXPERIMENT STATION, UNIVERSITY OF MAINE
ORONO, MAINE

LITERATURE CITED

1. BREED, ROBERT S., E. G. D. MURRAY, and A. PARKER HITCHENS. Bergey's manual of determinative bacteriology. 6th ed. The Williams & Wilkins Co., Baltimore, Maryland. 1948.
2. BUCHANAN, R. E., and R. ST. JOHN-BROOKS. Proposed bacteriological code of nomenclature. The Iowa State College Press, Ames, Iowa. 1947.
3. DETMERS, FREDA. A bacterial disease of the raspberry. Ohio Agr. Exp. Sta. Bul. (Second Ser.) 4. pp. 128–129. 1891.
4. ELLIOTT, CHARLOTTE. Manual of bacterial plant pathogens. 349 pp. Williams and Wilkins Co., Baltimore. 1930.
5. FOLSOM, DONALD. Bacterial twig and blossom blight of raspberry in Maine. U. S. Dept. Agr., Pl. Dis. Rptr. **31**: 324. 1947.
6. HEALD, F. D. Manual of plant diseases. 2nd ed. McGraw-Hill Book Co., New York. 1933.
7. LEHMAN, S. Bacterial twig and blossom blight of raspberry. (Abstr.) Phytopath. **23**: 21. 1933.
8. STARR, M. P. The causal agent of bacterial root and stem disease of guayule. Phytopath. **37**: 291–300. 1947.
9. ———. The nutrition of phytopathogenic bacteria. III. The Gram-positive phytopathogenic Corynebacterium species. Jour. Bact. **57**: 253–258. 1949.
10. ———, and W. H. BURKHOLDER. Lipolytic activity of phytopathogenic bacteria determined by means of spirit blue agar and its taxonomic significance. Phytopath. **32**: 598–604. 1942.
11. ———, and M. MANDEL. The nutrition of phytopathogenic bacteria. IV. Minimal nutritive requirements of the genus Erwinia. Jour. Bact. **60**: 669–672. 1950.
12. ———, and J. E. WEISS. Growth of phytopathogenic bacteria in a synthetic asparagin medium. Phytopath. **33**: 314–318. 1943.

HOST RANGE OF THE PIERCE'S DISEASE VIRUS OF GRAPES AS DETERMINED BY INSECT TRANSMISSION

J. H. FREITAG[1]

(Accepted for publication June 18, 1951)

Evidence obtained by Hewitt and Houston (4) indicated that in California the virus causing Pierce's disease of grapes and that causing alfalfa dwarf were identical. This suggested that other plants might be susceptible and that the virus might have a wide host range. In addition, the three important leafhopper vectors of Pierce's disease (5, 6, 10) virus were collected on a wide variety of host plant species, from grasses and sedges to shrubs and trees, further indicating the possibility of a wide virus host range and promising the necessary vectors in nature, capable of feeding on a variety of plants.

Because greenhouse work to determine the insect vectors had indicated that grape and alfalfa were not ideal host plants for the display of unmistakable symptoms of this virus, studies were undertaken to find more suitable hosts, both experimental and natural, to be used as test plants for diagnosis of the disease, as well as to identify the virus and to determine other possible economic host plants. Such knowledge would provide information on possible natural sources of the virus and aid materially in devising a control program.

MATERIALS AND METHODS

Insect vectors used for inoculations. Because of limitations of grafting, all inoculations were made by means of three species of leafhoppers, collected in the field. The majority of the herbaceous plants were inoculated by the green sharpshooter, *Draeculacephala minerva* Ball, and a few by the red-headed sharpshooter, *Carneocephala fulgida* Nott.; the blue-green sharpshooter, *Hordnia circellata* (Baker), was used for the shrubs and woody plants.

Green sharpshooters, collected usually in irrigated pastures, were fed for 1 or 2 days on alfalfa plants infected with dwarf before they were transferred to the various plant species being tested for susceptibility. In some tests they were fed on an infected grapevine, but such plants proved to be poor hosts and mortality was high even when the leafhoppers fed only 24 hr. The red-headed sharpshooter was likewise fed on dwarf alfalfa and only occasionally on diseased grape.

Blue-green sharpshooters collected mainly on ornamental shrubs at Berkeley and others obtained in their natural breeding areas along stream banks in Napa County were fed for 1–5 days on diseased grapevines before being transferred to healthy test plants.

[1] The assistance of N. W. Frazier, E. S. Sylvester, and W. N. Stoner in the work reported is gratefully acknowledged.

From 5 to 20 leafhoppers were used to inoculate a plant, depending on its size and the available supply of vectors. Normally the insects were allowed to feed until they died on the plants, but if the plant was a favorable host and eggs were deposited, the adult leafhoppers were removed before the eggs hatched. Some plants were poor hosts and the insects died after 1 or 2 days; on other species the insects survived for long periods but did not deposit eggs.

Rearing noninfective insects. A method devised to obtain noninfective insects for greenhouse tests could be relied upon to provide noninfective insects, but the resulting populations were usually too small to justify the amount of time and effort involved in the procedure. Many gravid female green sharpshooters were collected in permanent pastures and caged on mildew-resistant Sacramento barley in the greenhouse. These females

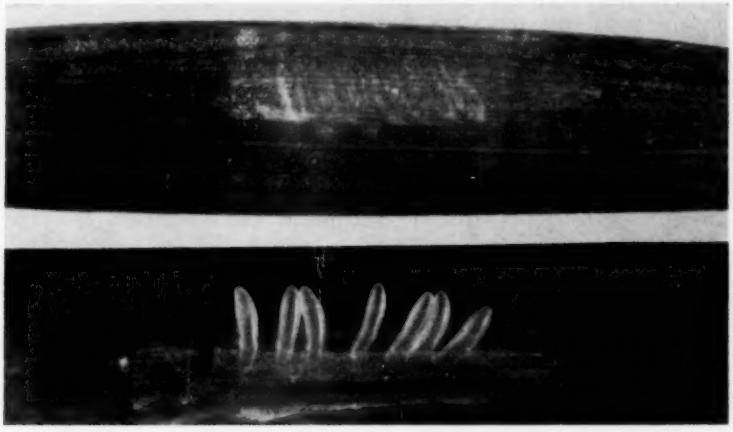

FIG. 1. Eggs of the green sharpshooter in leaf tissue of Sacramento barley: Above, in position with eye spots developing; below, partially exposed.

would usually deposit a large number of eggs in the barley leaf tissue, inserted with the long axes parallel in packets of 2 to 18, just below the epidermal surface of the leaf (Fig. 1). The resulting slightly raised epidermal areas are best seen by transmitted light.

After an incubation period of 10–12 days for development of the young embryo, the eye spots were well pigmented and dark. When the leaf epidermis was lifted with a dissecting needle under a binocular microscope, the eggs were readily dissected out and transferred to a moistened filter paper to complete their incubation period.

Newly born nymphs were allowed to crawl onto leaves of barley seedlings and to develop there for 2 to 4 weeks. During this period the nymphs had reached the third, fourth, or fifth instar. Since, as is shown later, the virus apparently does not pass through the egg, these nymphs were noninfective and could be used in tests to recover virus from inoculated host plants. A number of noninfective leafhoppers reared by this method were

successfully used to recover virus from either experimentally or naturally infected plants.

The blue-green sharpshooter deposits its eggs singly and not in groups as do the other two vector species; hence it was not practical to dissect the eggs from plant tissue. Noninfective insects were obtained by removing nymphs as they hatched and transferring them immediately to healthy grapevines, but this method was too slow and laborious to provide the large number of noninfective insects needed for experimental testing. Most of the recovery tests were made with the green sharpshooter, because of the greater ease of obtaining noninfectives of this species.

Numerous attempts to rear sharpshooter leafhoppers have failed, however, to give populations of sufficient numbers for use in experimental work. Even though many nymphs hatched from eggs of the field insects, usually only a few individuals would mature as adults. The females reared in greenhouse cages deposited very few eggs, and the resulting colonies were small. Many different plants were tested as possible rearing hosts, the various species of grasses being the most favorable. Apparently a number of factors necessary in the rearing of sharpshooters were not satisfied.

Test plants. Test plants used in the host range studies were alfalfa, *Medicago sativa* L., of the variety California Common or Chilean, grown from seed; and grape, principally European, *Vitis vinifera* L. The latter were rooted cuttings of field vines known to be free of the Pierce's disease virus and located in an area of relatively low prevalence of the disease. Danger of natural infection of the rootings obtained from field plants was always a factor, but control plants held for observation indicated only rare infection. The table grape variety, Emperor, was the most useful from the standpoint of symptom development in the greenhouse and was used in most of the tests. The varieties Thompson Seedless, Ribier, Palomino, and Malaga were used to a limited extent.

Seedlings of wild grape (*Vitis californica* Benth.) were used in some tests, but it was sometimes difficult to differentiate between symptoms resulting from Pierce's disease infection and those due to other causes. Seedlings from Emperor and Malaga grapes were also unreliable as to symptom development. The use of seedlings greatly reduced the possibilities of accidental infection, but because of this unreliability and their variation in vigor and in size and shape of leaves, it was concluded that vines derived from cuttings were on the whole more satisfactory for most tests.

Recovery of virus from experimentally infected plants. Of several methods to recover virus from inoculated plants, the one most often followed, when the host plant proved favorable to the insect, consisted of testing the nymphs that hatched from eggs deposited in the inoculated plants. This procedure was most successful with the various species of grasses but was useful in testing a number of plants. The adult leafhoppers used to inoculate the plants were removed before the nymphs hatched. After 2–4

weeks on the inoculated plants the nymphs were transferred to healthy grape and alfalfa test plants, where they fed for one week and then were removed.

If the plant being tested was an unfavorable host for egg deposition, introduced noninfective insects were fed on it to determine whether it had become infected with the virus. Since efforts to rear the leafhoppers in the greenhouse had yielded only low populations, recovery tests were attempted with field leafhoppers believed to be noninfective, collected in areas that were relatively free of Pierce's disease virus. The results, however, indicated that practically all field populations tested contained individual leafhoppers already carrying the virus. Thus the use of field insects for recovering virus from experimentally infected plants was unsatisfactory and such insects were used only when no other method of testing was possible. For a check on their infectivity, the field-collected sharpshooters were fed on healthy grape or alfalfa plants before use in recovery tests: if the plants developed symptoms of the virus, the results of such tests were disregarded.

Recovery of virus from naturally infected plants. Three methods were used to recover virus from plants suspected of being naturally infected with the Pierce's disease virus: 1. Plants (mainly grasses) growing under natural conditions which had leafhopper eggs deposited within leaf tissues were dug up and transplanted to flowerpots in the greenhouse. Each plant was covered with a cage and the eggs were allowed to hatch. If the plants were in good condition, the nymphs were permitted to develop for 2–4 weeks. Nymphs in the third, fourth, and fifth instar were then transferred, in groups of 10 to 20, in equal numbers to healthy grape and alfalfa plants. This method was most successfully used in testing various species of grasses for the presence of natural infection. 2. Nymphs of the blue-green sharpshooter were collected from suspected plants and transferred to healthy grape and alfalfa plants. This procedure was followed mainly for hosts of this sharpshooter, chiefly perennial plants such as shrubs and trees. Care was exercised in selecting such plants, so as to be relatively certain the nymphs had not moved in from other host plants. 3. Greenhouse-reared noninfective leafhoppers were fed on shoots of plants suspected of being naturally infected with the virus, and transferred to healthy grape and alfalfa plants.

SYMPTOMS

Results of host range studies indicate that a large number of plants are susceptible to Pierce's disease virus, but that very few plants other than grape and alfalfa develop symptoms of the disease. Many of these symptomless carriers are important sources of virus in the natural spread of the disease.

The first symptom in the greenhouse on grape cuttings used as test plants consisted of reddish discoloration and burn of dentations along leaf margins (Fig. 2, left), subtended by a band of reddish tissue. The burned

area gradually widened, with an adjacent band of discolored tissue and an inner chlorotic area advancing ahead of it (Fig. 2, right). Finally the entire leaf became necrotic. The canes matured irregularly, as evidenced by green patches in the bark. Grape cuttings infected with the virus usually died several months after infection.

Four species of clover developed definite symptoms of the disease: Hubam clover, *Melilotus alba* Desr. var. *annua* Coe; white sweet clover, *M. alba* Desr.; yellow sweet clover, *M. officinalis* Lam.; and annual yellow sweet clover, *M. indica* All. Leaflets yellowed, especially along the margins, and

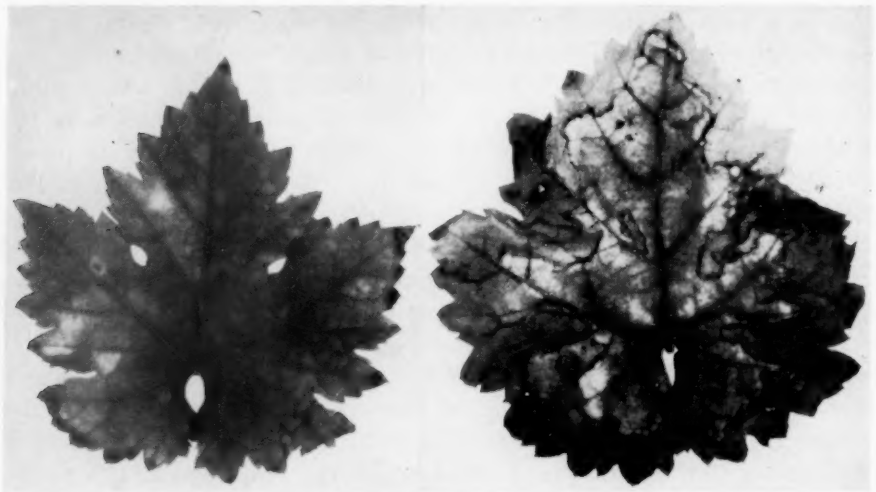

FIG. 2. Leaf burn of Emperor grape caused by Pierce's disease virus. Left, early stage; reddish discoloration of marginal dentations and beginning of marginal burn. Right, advanced stage; entire marginal area necrotic, reddish discoloration and chlorotic zone subtending the burned area.

a marginal burning (Fig. 3, A) gradually involved the entire leaflet. Leaves were dwarfed, curled upward, and borne on reduced stems (Fig. 3, B). The plants also were stunted and the prematurely dried leaflets dropped, leaving the petioles still attached to the main stem. In roots of clover a brown discoloration of the vascular tissue resembled that described by Weimer (9) for alfalfa.

Snowberry, *Symphoricarpos albus* (L.), was very susceptible to the virus and every one of the six plants inoculated by feeding infective blue-green sharpshooters became infected: leaves became chlorotic and marginally burned, then dried, and finally the entire plant died.

A number of plants such as willow herb, *Epilobium californicum* Hausskn.; California mugwort, *Artemisia vulgaris* L. var. *heterophylla* Jepson; and creek nettle, *Urtica gracilis* Ait. var. *holosericea* Jepson were very susceptible to the virus but developed no symptoms in the greenhouse that could be definitely attributed to the virus. No symptoms were detected on

FIG. 3. A. Hubam clover leaves with chlorosis, marginal burn, and tip burn caused by Pierce's disease virus. (Normal green leaf from healthy plant at upper left.) B. Terminal shoots of white sweet clover: Left, stunted, chlorotic terminal shoot with dwarfed, curled, burned leaves, from plant infected with Pierce's disease virus. Right, shoot from normal healthy plant.

TABLE 1.—*Plant species experimentally infected with Pierce's disease virus as proved by recovery of the virus by the insect vectors*

Host plant	Inoculated plants[a]	Method of virus recovery	Test plants[b]	
			Alfalfa	Grape
Gramineae, Grass Family				
Wild oat, *Avena fatua* L.	3/5	Nymphs hatched[c]	3/3	1/5
Rescue grass, *Bromus catharticus*	4/5	do	2/10	3/10
Russian brome grass, *Bromus* sp.	1/5	do	0/0	1/9
Ripgut grass, *B. rigidus* Roth	8/15	do	9/20	3/20
Bermuda grass, *Cynodon dactylon* (L.) Pers.	14/29	do	10/25	12/25
		Noninfectives[d]	2/9	1/9
Hairy crabgrass, *Digitaria sanguinalis* (L.) Scop.	3/15	Nymphs hatched	1/11	2/14
Barnyard grass, *Echinochloa crusgalli* (L.) Beauv.	12/24	do	2/18	5/29
Diffuse love grass, *Eragrostis diffusa* Buckl.	8/10	do	5/13	9/13
Johnson grass, *Holcus halepensis* L.	5/24	do	3/30	2/30
Sudan grass, *H. sudanensis* Bailey	1/5	do	1/9	0/11
Common foxtail, *Hordeum murinum* L.	4/5	do	4/5	2/5
Barley, *H. vulgare* L.	4/8	do	1/5	3/9
Foxtail fescue, *Festuca megalura* Nutt.	1/4	do	0/3	1/1
Dallis grass, *Paspalum dilatatum* Poir.	2/2	Noninfectives	0/2	2/2
Mediterranean canary grass, *Phalaris minor* Retz	3/15	Nymphs hatched	2/21	1/16
Gnawed canary grass, *P. paradoxa* L.	4/5	do	6/8	4/7
Timothy, *Phleum pratense* L.	2/3	do	2/3	1/2
Annual bluegrass, *Poa annua* L.	1/2	do	1/2	0/0
Kikuyu grass, *Pennisetum clandestimum* Hochst.	1/5	do	0/5	1/5
Italian rye grass, *Lolium multiflorum* Lam.	10/15	do	11/12	14/17
Darnel, *L. temulentum* L.	5/5	do	8/8	5/9
Yellow bristlegrass, *Setaria lutescens* (Weigel) F. T. Hubb	7/19	do	5/24	2/22
Cyperaceae, Sedge Family				
Yellow nutgrass, *Cyperus esculentus* L.	3/5	Nymphs hatched	0/0	3/8
Cannaceae, Canna Family				
Canna sp.	2/2	Noninfectives	1/2	2/2
Urticaceae, Nettle Family				
Creek nettle, *Urtica gracilis*, Ait. var. *holosericea* Jepson	7/8	Nymphs hatched	2/3	6/7
Polygonaceae, Buckwheat Family				
Black bindweed, *Polygonum convolvulus* L.	1/2	Noninfectives	1/2	1/2
Lady's thumb, *P. persicaria* L.	3/7	do	2/7	2/7
Curly dock, *Rumex crispus* L.	1/5	Nymphs hatched	1/4	1/4
Rhubarb, *Rheum rhaponticum* L.	1/5	do	1/5	0/5
Chenopodiaceae, Goosefoot Family				
Mexican tea, *Chenopodium ambrosioides* L.	2/11	Nymphs hatched	1/10	1/11
Resedaceae, Mignonette Family				
Common mignonette, *Reseda odorata* L.	1/1	do	1/1	0/1
Pittosporaceae, Pittosporum Family				
Karo, *Pittosporum crassifolium* Cunn.	1/3	Noninfectives	0/3	1/3
Rosaceae, Rose Family				
Toyon or Christmas berry, *Photinia arbutifolia* Lindl.	1/4	do	1/4	1/4
California blackberry, *Rubus vitifolius* C. & S.	2/10	Nymphs hatched	2/9	1/9
Cotoneaster, *Cotoneaster rotundifolia Wall.* var. *lanata* Schneid.	1/1	do	0/0	1/1
Leguminosae, Pea Family				
Scotch Broom, *Cytisus scoparius* Link	1/3	Field insects[e]	1/3	1/3
Lathyrus cicera	4/5	Noninfectives	4/5	4/5
L. clymenium	5/5	do	7/9	6/6
Grass pea, *L. sativa* L.	5/5	do	5/5	5/5
White melilot, *Melilotus alba* Desr.	4/4	do	3/4	4/4
Hubam clover, *M. alba* Desr. var. *annua* Coe	5/5	Field insects	4/5	5/5
Annual yellow sweet clover, *M. indica* All.	4/9	Noninfectives	4/9	3/9
	4/5	Nymphs hatched	3/5	4/4
Yellow sweet clover, *M. officinalis* Lam.	2/3	Noninfectives	2/3	2/3

Host plant	Inoculated plants[a]	Method of virus recovery	Test plants[b]	
			Alfalfa	Grape
Strawberry clover, *Trifolium fragerum*	1/1	Nymphs hatched	0/0	1/1
Alsike clover, *T. hybridum* L.	5/5	Field insects	2/5	4/5
Crimson clover, *T. incarnatum* L.	3/3	do	3/3	2/3
Red clover, *T. pratense* L.	5/5	Nymphs hatched	5/5	0/0
White clover, *T. repens* L.	5/5	Field insects	4/5	5/5
Ladino clover, *T. repens* L. var. *latum* McCarthy	3/7	Noninfectives	2/6	3/7
Vetch, *Vicia monanthus*	4/5	do	4/5	0/1
Geraniaceae, Geranium Family				
Red-stem filaree, *Erodium cicutarium* L'Her.	3/5	Nymphs hatched	1/6	2/5
Fish geranium, *Pelargonium hortorum* Bailey	2/2	do	0/1	2/2
Vitaceae, Vine Family				
Boston ivy, *Parthenocissus tricuspidata* Planch.	1/6	Field insects	0/0	1/6
Myrtaceae, Myrtle Family				
Australian brush-cherry, *Eugenia myrtifolia* Sims	1/4	do	0/0	1/4
Onagraceae, Evening Primrose Family				
Evening primrose, *Oenothera hookeri* T. & G.	5/18	Nymphs hatched	2/14	3/16
Godetia, *Godetia grandiflora* Lindl.	1/4	do	2/4	0/0
Willow herb, *Epilobium californicum* Hausskn.	7/14	do	1/14	7/15
Panicled willow herb, *Epilobium paniculatum* Nutt.	2/4	Field insects	3/4	0/0
Araliaceae, Aralia Family				
Variegated ivy, *Hedera helix* L.	2/2	Noninfectives	1/2	1/2
Umbelliferae, Parsley Family				
Short white carrot, *Daucus carota* L. var. *sativa* DC.	2/2	Nymphs hatched	2/2	0/0
Water parsley, *Oenanthe sarmetosa* Presl.	3/6	do	0/1	3/9
Oleaceae, Olive Family				
Lilac, *Syringa vulgaris* L.	**1/1**	Noninfectives	1/1	0/1
Apocynaceae, Dogbane Family				
Periwinkle, *Vinca major* L.	2/5	Field insects	0/0	2/5
Boraginaceae, Borage Family				
Buckthorn weed, *Amsinckia douglasiana* DC.	2/2	Nymphs hatched	1/2	1/1
Labiatae, Mint Family				
Mint, *Mentha* sp.	1/1	do	0/0	2/2
Rubiaceae, Madder Family				
Coprosma baueri Endl.	4/4	do	2/3	4/4
Caprifoliaceae, Honeysuckle Family				
Blue elder, *Sambucus caerulea* Raf.	7/14	do	4/14	5/20
Japanese honeysuckle, *Lonicera japonica* Thunb.	4/5	do	4/4	1/4
Snowberry, *Symphoricarpos albus* (L.) Blake	6/6	do	3/3	5/6
Compositae, Sunflower Family				
China aster, *Callistephus chinensis* Nees.	1/4	do	0/0	1/6
Annual bur-weed, *Franseria acanthicarpa* (Hook.) Cov.	1/5	do	0/4	1/4
California mugwort, *Artemisia vulgaris* L. var. *heterophylla* Jepson	3/5	do	2/4	11/17
Prickly lettuce, *Lactuca scariola* L.	1/5	Noninfectives	1/5	0/5
Prickly sowthistle, *Sonchus asper* (L.) Hill	1/5	do	0/4	1/4
Cocklebur, *Xanthium canadense* Mill.	4/7	Nymphs hatched	2/5	3/7

[a] Numerator, number of inoculated plants from which virus was recovered; denominator, number of inoculated plants tested.

[b] Numerator, number of test plants infected; denominator, number of test plants inoculated.

[c] Infective leafhoppers deposited eggs in the inoculated plants; nymphs hatched from these were transferred to grape and alfalfa plants.

[d] Greenhouse-reared leafhoppers from dissected eggs. These were fed on inoculated plants and then transferred to grape and alfalfa test plants.

[e] Collected under natural conditions, tested for infectivity, and proved to be noninfective. These were fed on inoculated plants, then transferred to grape and alfalfa test plants.

any species of the grasses tested, yet they proved to be among the best sources of the virus for the sharpshooter vectors.

HOST RANGE AS DETERMINED BY EXPERIMENTAL INFECTION

Pierce's disease virus was experimentally transmitted to and recovered from 75 species of plants belonging to 23 families (Table 1) by various methods. Experimental infection was demonstrated in plants of 49 species by transferring nymphs which hatched from them to grape and alfalfa indicator plants; results show not only the wide host range of the virus, but also the wide list of host plants favorable for development of the insect vectors. Noninfective insects recovered the virus from 19 species and field-collected noninfective insects recovered it from nine species. The error, which may have occurred through use of field insects, is not considered significant.

Grasses of 22 species were experimentally infected with Pierce's disease virus, and were also favorable food and breeding plants of the sharpshooters. Certain grasses play an important and dual role in the spread of virus by supplying both the virus source and the insect population necessary for the spread of virus; Bermuda grass, for example, is one of the most favorable hosts for the development of sharpshooters, and is also a virus host. The grasses should, consequently, be given important consideration in any control program attempted.

The green sharpshooter, *Draeculacephala minerva* (Ball), was used for most of the inoculations and was relatively efficient in infecting grasses and recovering virus from this group of plants. Bermuda grass was infected with difficulty by this vector and recovery trials from 11 inoculated plants resulted in the infection of only four of 12 grapes and two of 12 alfalfa plants. The red-headed sharpshooter, *Carneocephala fulgida* (Nott.), apparently is somewhat more efficient in the transmission of virus to Bermuda grass; it infected eight of 18 grape and seven of 18 alfalfa plants in recovery tests made from 10 inoculated plants.

Clovers and other legumes were among the plants most susceptible to the virus, and 13 species were experimentally infected. Legumes were good virus sources from which leafhoppers readily recovered the virus. Although the clovers were good food plants for survival of the leafhoppers, only a few of them were favorable hosts for their reproduction.

Pierce's disease virus was common in ornamental plants usually grown in home gardens. Sixteen species were experimentally infected with the virus. None of these plants developed any definite virus symptoms either in the garden or in the greenhouse. The blue-green sharpshooter was especially abundant in some of these gardens in the coastal areas, particularly at Berkeley and Los Angeles and in the Napa Valley. Because nymphs taken from ornamental shrubs and trees were shown to be carrying the virus, plants on which they were collected apparently were infected. In

addition to the perennials, a few annual plants grown as ornamentals were experimentally infected.

Ornamental gardens consisting mainly of shrubs and trees constitute a disease hazard in their high populations of infective leafhoppers, when located adjacent to vineyards.

Weeds other than grasses were also a source of virus for the leafhoppers. Nineteen species of weeds, including a sedge, were experimentally infected with the virus. The various weeds are also a source of infective leafhopper populations and hence a hazard to near-by vineyards.

Among the cultivated plants, 21 species were experimentally infected. Important economic crop plants found susceptible were grape, alfalfa, carrots, clover, and vetch. Only grape and alfalfa have been known to suffer losses from Pierce's disease virus.

NATURALLY INFECTED PLANT SPECIES

Thirty-six species of plants belonging to 18 families were demonstrated to be naturally infected with the Pierce's disease virus (Table 2). The virus was recovered from seven of these by hatching nymphs from eggs deposited under natural conditions; from 12 by means of previously noninfective sharpshooters; and from 18 by the use of nymphs carefully collected under natural conditions on host plants having high populations of the leafhoppers. Plant species naturally infected with the virus included eight grasses, nine ornamentals, and 13 weeds.

There were no symptoms by which naturally infected plants, other than grape and alfalfa, could be recognized. Only with high leafhopper populations would there be a reasonable possibility of selecting an infected plant. Thus the discovery of natural hosts of the virus depended somewhat on the chance selection of an infected plant among many which were probably not diseased.

PLANT SPECIES WHICH FAILED TO DEVELOP INFECTION

The following plants were experimentally inoculated with Pierce's disease virus but did not develop symptoms, and noninfective sharpshooter vectors failed to recover virus from them. Additional tests might show some of these plants to be susceptible to the virus.

Gramineae: saltgrass, *Distichlis spicata* (L.) Greene; Kentucky bluegrass, *Poa pratensis* L.; rabbitfoot grass, *Polypogon monspeliensis* Desf.

Pinaceae: Douglas fir, *Pseudotsuga taxifolia* Britt.

Salicaceae: cottonwood, *Populus* sp.

Chenopodiaceae: sugar beet, *Beta vulgaris* L.

Aizoaceae: New Zealand spinach, *Tetragonia expansa* Murr.

Caryophyllaceae: common purslane, *Portulaca oleracea* L.

Calycanthaceae: spicebush, *Calycanthus occidentalis* H. & A.

Cruciferae: common yellow mustard, *Brassica campestris* L.

TABLE 2.—*Plant species naturally infected with Pierce's disease virus as proved by recovery of the virus by the insect vectors*

Host plant	Recovery of the virus		Test plants[b]	
	No. of plants[a]	Method	Alfalfa	Grape
Gramineae, Grass Family				
Bermuda grass, *Cynodon dactylon* (L.) Pers.	6/38	Nymphs hatched[c]	3/15	3/10
Ripgut grass, *Bromus rigidus* Roth.	4/43	do	2/14	2/13
Italian ryegrass, *Lolium multiflorum* Lam.	4/6	Noninfectives[d]	3/5	1/5
Dallis grass, *Paspalum dilatatum* Poir.	3/4	do	1/4	2/6
Wild oats, *Avena fatua* L.	12/59	Nymphs hatched	4/29	11/30
Annual bluegrass, *Poa annua* L.	1/1	do	0/0	1/1
Hairy crabgrass, *Digitaria sanguinalis* (L.) Scop.	7/21	do	0/18	7/19
Barnyard grass, *Echinochloa crusgalli* (L.) Beauv.	5/21	do	0/13	6/15
Salicaceae, Willow Family				
Willow, *Salix* sp.	2/10	Nymphs collected[e]	0/0	2/10
Urticaceae, Nettle Family				
Creek nettle, *Urtica gracilis* Ait. var. *holosericea* Jepson	5/13	do	0/0	5/13
Polygonaceae, Buckwheat Family				
Curly dock, *Rumex crispus* L.	1/5	Noninfectives	1/5	0/5
Lady's thumb, *Polygonum persicaria* L.	2/2	do	2/2	0/2
Chenopodiaceae, Saltbush Family				
Mexican tea, *Chenopodium ambrosioides* L.	1/3	do	0/3	1/3
Saxifragaceae, Saxifrage Family				
Escallonia montevidensis DC.	1/1	do	1/1	0/1
Hydrangea paniculata Sieb.	2/5	Nymphs collected	0/0	2/5
Rosaceae, Rose Family				
California blackberry, *Rubus vitifolius* C. & S.	1/1	Noninfectives	1/1	0/0
	4/10	Nymphs collected	0/0	4/10
California wild rose, *Rosa californica* C. & S.	5/10	do	0/0	5/10
Leguminosae, Pea Family				
Sydney golden wattle, *Acacia longifolia* Willd.	2/2	Noninfectives	1/3	1/3
Scotch broom, *Cytisus scoparius* Link	1/1	do	0/0	1/1
Sweet clover, *Melilotus* sp.	3/4	do	1/5	2/6
Bur clover, *Medicago hispida* Gaertn.	1/1	do	1/5	1/5
Ladino clover, *Trifolium repens* L. var. *latum* McCarthy	2/5	Noninfectives	1/5	1/5
Anacardiaceae, Sumac Family				
Poison oak, *Rhus diversiloba* T. & G.	1/1	Nymphs hatched	0/0	1/1
Vitaceae, Vine Family				
Boston ivy, *Parthenocissus tricuspidata* Planch	3/13	Nymphs collected	1/5	2/8
California wild grape, *Vitis californica* Benth.	4/10	do	0/0	4/10
Myrtaceae, Myrtle Family				
Australian brush-cherry, *Eugenia myrtifolia* Sims	4/10	do	0/0	4/10
Onagraceae, Evening Primrose Family				
Fuchsia, *Fuchsia magellanica* Lam.	4/5	do	0/0	4/5
Oleaceae, Ash Family				
Foothill ash, *Fraxinus dipetala* H. & A.	3/7	do	0/0	4/12
Verbenaceae, Vervain Family				
Pigeon-berry, *Duranta repens* L.	2/5	do	0/0	2/5
Labiatae, Mint Family				
Rosemary, *Rosmarinus officinalis* L.	2/12	Nymphs collected	0/0	2/12
Sweet Marjoram, *Majorana hortensis* Moench	1/5	do	0/0	1/5
Garden balm, *Melissa officinalis* L.	6/10	do	0/0	6/10
Scrophulariaceae, Figwort Family				
Speedwell, *Veronica* sp.	1/5	do	0/0	1/5
Capriofoliaceae, Honeysuckle Family				
Blue elder, *Sambucus caerulea* Raf.	4/10	do	0/0	4/10
Compositae, Sunflower Family				
California mugwort, *Artemisia vulgaris* L. var. *heterophylla* Jepson	4/12	do	0/0	4/12
Coyote brush, *Baccharis pilularis* DC.	3/5	do	1/5	2/5

Saxifragaceae: syringa, *Philadelphus lewisii* Pursh var. *californicus* Gray.

Rosaceae: apple, *Malus sylvestris* Mill.; Japanese apricot, *Prunus mume* Sieb & Zuce; western chokecherry, *Prunus demissa* (Nutt.) Dietr.; firethorn, *Pyracantha angustifolia* Schneid.; *Cotoneaster francheti* Bois.

Rutaceae: Meyer lemon, *Citrus limon* Burm.; tangerine, *Citrus reticulata* Blanco.

Sapindaceae: buckeye, *Aesculus californica* (Spach) Mutt.

Malvaceae: cheeseweed, *Malva parviflora* L.

Scrophulariaceae: bush monkeyflower, *Diplacus aurantiacus* Jepson.

Acanthaceae: English plantain, *Plantago lanceolata* L.

Compositae: pineapple weed, *Matricaria suaveolens* (Pursh) Buch.; bur marigold, *Bidens pilosa* L.; wild sunflower, *Helianthus* sp.

EVIDENCE THAT THE VIRUS IS NOT TRANSMITTED THROUGH THE EGG OF ITS INSECT VECTOR

Viruses transmitted by leafhoppers are generally not transmitted through the egg of the insect to its offspring. There are, however, two exceptions to this rule, rice dwarf virus (3) and the clover club-leaf virus (1), both of which are transmitted through the eggs of infective leafhoppers. Since the fundamental basis for the host range results reported is dependent on the assumption that the nymphs are noninfective when they hatch from the egg, it became important to investigate this point.

In order to determine whether Pierce's disease virus is transmitted through the egg of the green sharpshooter, a number of tests were made. Female sharpshooters collected in irrigated pastures were allowed to deposit eggs in barley tissue, and after a 10-day incubation period the eggs were cut from the tissue. There was less mortality under these conditions than when eggs were dissected earlier in their development. The reason for removing the eggs was to prevent the young nymphs from obtaining virus from the barley plant, which may have been infected by the adult insects. The eggs were placed on moist filter paper in small vials and allowed to hatch. The young nymphs were allowed to crawl onto young barley seedlings and develop for 2–3 weeks. The third and fourth instar nymphs were transferred for testing to healthy grape and alfalfa plants.

In the first series of tests the field-collected sharpshooters were transferred directly to barley without determining their natural infectivity and without feeding them on diseased plants to insure that they were infective.

[a] Numerator, number of plants from which virus was recovered; denominator, number of plants tested.

[b] Numerator, number of plants infected; denominator, number of plants inoculated.

[c] Infective leafhoppers deposited eggs in the inoculated plants; nymphs hatched from these were transferred to grape and alfalfa plants.

[d] Greenhouse-reared leafhoppers from dissected eggs. These were fed on inoculated plants and then transferred to grape and alfalfa test plants.

[e] Nymphs were collected on host plants suspected of being naturally infected, and transferred to grape and alfalfa test plants. Care was exercised so that it could be reasonably certain that nymphs had hatched from the plants on which they were found.

In a second series the field-collected sharpshooters were first fed on diseased grape plants and then tested for ability to transmit the virus to healthy plants before being transferred to barley for egg deposition. The results of both series were negative and indicated that the Pierce's disease virus is not transmitted through the egg of the insect vector. The nymphs on hatching from the eggs are consequently noninfective and must obtain virus by feeding on infected host plants before they are capable of transmitting it to healthy plants.

DISCUSSION

Among leafhopper-transmitted viruses that have been demonstrated to have wide host ranges are those of aster yellows (8), curly top (2), tomato big bud (7), and Pierce's disease. These viruses are all transmitted by grafting and by means of the insect vector, but not by mechanical inoculation. The host ranges were determined by feeding infective leafhopper vectors on a large variety of plants. The curly top, aster yellows, and tomato big bud viruses all developed distinct and pronounced symptoms on the various susceptible species, and the host ranges of these viruses were primarily based on the production of symptoms. Pierce's disease virus, however, developed symptoms on relatively few host plants. The work with this virus was, therefore, based not on the production of symptoms but on the ability of leafhoppers to recover virus from symptomless plants.

The curly top and Pierce's disease viruses were readily recovered from infected plants by means of leafhoppers, but the aster yellows virus was recovered from certain susceptible plants with difficulty. Kunkel (8) determined susceptibility to aster yellows virus mainly on the development of symptoms. In the work on Pierce's disease it was necessary to feed noninfective insects on the inoculated plants and to transfer these to grape and alfalfa test plants in order to ascertain whether the inoculated plants were infected with the virus. The fact that the sharpshooter vectors could be reared only in small numbers made this problem of virus recovery tests a difficult one.

The results of the tests indicate that the Pierce's disease virus has a wide host range. There are, however, undoubtedly other susceptible species which were not tested. Most species were selected at random; others were chosen because they were known hosts of the insect vectors. The virus is certainly not limited in its distribution because of lack of susceptible species in nature. The fact that there was usually a high incidence of Pierce's disease virus adjacent to alfalfa fields, pastures, ditch banks, and roadsides strongly indicated that plants other than grape and alfalfa were hosts of the virus. The information on the host range was needed in order to devise control methods and to determine relationship of the Pierce's disease virus to other known viruses.

There apparently is no way of predicting the susceptibility of a plant to a virus. The ability of a plant to support the increase of a virus in its

tissues is characteristic of the species and may have little or no relationship to the taxonomic position of the plant.

There appears to be no correlation between the host range of a virus and the host range of its leafhopper vectors. Many preferred host plants of the leafhopper may not be susceptible to the virus and plants which are unfavorable to the insect may be very susceptible to the virus.

Though a virus may infect a considerable number of species, it must not be considered totally lacking in host plant specificity. Given an adequate number of tests, it usually can be demonstrated that a large number of species may be immune from the virus. Since the problems involved in conducting host range studies with the Pierce's disease virus were so difficult, no attempt was made to demonstrate which hosts might be immune. Other species that have resisted virus infection up to the present time may succumb under more favorable environmental conditions.

The possibility of confusing symptoms of alfalfa dwarf and other diseases of the alfalfa plant was constantly a factor. While browning of the cortical area of the root was typical and unmistakable on a great majority of infected alfalfa plants, milder symptoms on other plants made it difficult to decide whether a plant was actually infected with the virus. Although many grape plants developed definite symptoms of the virus in the greenhouse, others had leaf burning which was hard to tell from burning caused by sunscorch, insect injury, or spray injury.

The disadvantages of grape and alfalfa as test plants for the Pierce's disease virus constituted one of the original reasons for investigating additional host plants which might develop more definite symptoms of the virus. Many annual plants were inoculated with the virus by leafhoppers in the hope of discovering one which could be grown from seed and would also develop definite symptoms in a relatively short period of time. The work failed to reveal any such host and it is now believed that it will be difficult to find a host plant of such description.

SUMMARY

The Pierce's disease virus was experimentally transmitted to 75 species of plants belonging to 23 plant families. Inoculations were made by means of three species of leafhoppers, *Draeculacephala minerva* Ball, *Hordnia circellata* (Baker), and *Carneocephala fulgida* Nott. Proof of infection depended on recovery of the virus by previously noninfective leafhoppers and its transfer to alfalfa and grape test plants.

The new host plants thus discovered include Johnson grass, Bermuda grass, rye grass, timothy, canna, toyon, Scotch broom, Hubam clover, alsike clover, crimson clover, red clover, Ladino clover, carrot, periwinkle, mint, vetch, and species of *Oenothera, Godetia, Hedera, Coprosma, Sambucus, Lonicera, Symphoricarpos,* and *Artemisia.*

Thirty-six species of plants belonging to 18 families were demonstrated to be naturally infected with the Pierce's disease virus. These include

Bermuda grass, Dallis grass, California blackberry, acacia, Boston ivy, rosemary, fuchsia, Ladino clover, and species of *Sambucus, Eugenia, Artemisia,* and *Baccharis.*

Symptoms of the Pierce's disease virus developed on only a few species of plants. The great majority of plants susceptible to the virus were symptomless carriers.

Thirteen species of legumes were experimentally infected and these were among the few susceptible plants which developed symptoms of the virus.

The grasses were readily susceptible and 22 species were experimentally infected. Eight species were found naturally infected with the virus. A species of sedge and 19 species of weeds were susceptible.

Sixteen species of plants grown as ornamentals were experimentally infected. Nine species were naturally infected with the virus.

Noninfective insects were obtained by dissecting eggs from leaf tissue of barley plants.

Pierce's disease virus apparently is not transmitted through the egg of the green sharpshooter.

Attempts to rear the leafhopper vectors of Pierce's disease virus resulted in the development of only a few individuals. No populations of sufficient numbers for extensive experimental work were reared.

DIVISION OF ENTOMOLOGY AND PARASITOLOGY, UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BLACK, L. M. Multiplication of clover club-leaf virus (Aureogenus clavifolium) in its insect vector. (Abstr.) Phytopath. **39**: 2–3. 1949.
2. FREITAG, JULIUS H., and HENRY, H. P. SEVERIN. Ornamental flowering plants experimentally infected with curly top. Hilgardia **10**: 263–302. 1936.
3. FUKUSHI, TEIKICHI. Retention of virus by its insect vectors through several generations. Proc. Imp. Acad. Tokyo **15**: 142–145. 1939.
4. HEWITT, WM. B., and BYRON R. HOUSTON. Association of Pierce's disease of grapevines and alfalfa dwarf in California. U. S. Dept. Agr., Pl. Dis. Reptr. **25**: 475–476. 1941.
5. ———, NORMAN W. FRAZIER, H. E. JACOB, and J. H. FREITAG. Pierce's disease of grapevines. Calif. Agr. Exp. Sta. Cir. 353. 1942.
6. ———, BYRON R. HOUSTON, NORMAN W. FRAZIER, and J. H. FREITAG. Leafhopper transmission of the virus causing Pierce's disease of grape and dwarf of alfalfa. Phytopath. **36**: 117–128. 1946.
7. HILL, A. V. Insect transmission and host plants of virescence (big bud of tomato). Jour. Coun. Sci. Ind. Res. (Aust.) **16**: 85–90. 1943.
8. KUNKEL, L. O. Studies on aster yellows in some new host plants. Contrib. Boyce Thompson Inst. **3**: 85–123. 1931.
9. WEIMER, J. L. Effect of the dwarf disease on the alfalfa plant. Jour. Agr. Res. [U. S.] **55**: 87–104. 1937.
10. WINKLER, A. J. Pierce's disease investigations. Hilgardia **19**: 207–264. 1949.

REPORT AND ABSTRACTS OF THE THIRTY-THIRD ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 33rd annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of Southern California, Los Angeles, California, June 19–21, in conjunction with the annual meeting of the Pacific Division of the A.A.A.S. Approximately 100 persons attended the meeting; Arizona, California, Hawaii, Idaho, Oregon, Utah, and Washington were represented. Included on the program were 48 papers, a demonstration session, a joint symposium on "Air Pollution and Plant Life," a banquet, and a field trip.

Officers elected for 1951–1952 were: *President*, WM. B. HEWITT; *Vice-President*, E. K. VAUGHAN. Other officers for 1951–1952, elected for a 2-year term last year, are: *Councilor*, S. M. DIETZ; and *Secretary-Treasurer*, GEORGE A. ZENTMYER.

GEORGE A. ZENTMYER, *Secretary-Treasurer.*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Some experiments on curing and dipping gladiolus corms. BALD, J. G. The uncertainty of results from dipping gladiolus corms to control corm-borne diseases led to a study of interactions between the condition of the corms and the fungicidal efficiency of the dips. The corms' natural barriers against pathogens, periderm and cuticle, were encouraged to develop ahead of invading organisms by curing corms at 35° C. immediately after digging. Lesions in the treated corms were initiated mainly by Fusarium and Botrytis; Penicillium and bacteria were also generally present when the lesions were in a relatively inactive state. Two Penicilliums capable of extending corm lesions were isolated: one acting slowly at about 5° C.; the other, at about 35° C. Two fungicidal materials, Lysol and ferbam, were tested because of their contrasting properties. Lysol at times decreased disease symptoms and increased yield, but at other times tended to increase disease and decrease yield. Suppression by Lysol of secondary invaders or of possible antagonists of Fusarium may explain these opposite effects. Ferbam apparently acted directly on Fusarium and not on secondary invaders. Each fungicide reduced expansion of some types of lesion and not of others. These effects were reflected in yield of corms. Best results for practical control of corm-borne diseases were obtained from curing corms at 35° C. immediately after digging, and use of a fungicidal dip before planting, in this case Lysol plus ferbam.

Differentiation of some tobacco mosaic strains occurring naturally in tobacco. BALD, J. G., AND MARION HAMMACK. Infection studies were made with samples of purified tobacco mosaic virus (TMV) previously characterized by their electrophoretic mobility. A major and a minor component of TMV from tobacco had been recognized by their different mobilities, and the latter was concentrated by means of this difference. Single-lesion isolates with the same mobility as the major component caused severe symptoms; single-lesion isolates with the same mobility as the minor component caused mild symptoms. These two kinds of TMV probably correspond to Johnson's severe and mild strains screened out by differential host reactions. From amongst the severe isolates with similar mobilities, two types were separated, one characterized by the usual severe mottle of TMV on tobacco, the other by severe distortion at certain stages in the growth of infected tobacco plants. The severe mottle types caused necrosis on a large proportion of inoculated plants of *Nicotiana affinis*, the distorting types little or no necrosis. Severe strains of both kinds characteristically produce yellow mutants. A yellow isolate obtained pure or nearly pure, on inoculation to tobacco, was translocated with difficulty through larger plants. Such a lack of invasiveness probably explains why this and other yellow mutants fail to compete in tobacco plants with the parent strains.

Vein yellowing caused by a mutant of the curly-top virus. BENNETT, C. W. In 1943 leaves with conspicuously yellowed veins were noted on one side of a Turkish to-

bacco plant that had recovered from curly top. The yellowing proved to be caused by a virus transmissible by the beet leafhopper, *Circulifer tenellus*, and by dodder, *Cuscuta californica*. The virus has not been separated from curly-top virus but the two have been transmitted in combination to sugar beet, tobacco, and tomato. No symptoms were observed on sugar beet but virus was recovered from inoculated plants. A golden yellow color is characteristic of affected tobacco plants, and the presence of the vein-yellowing virus results in a marked decrease in concentration of the curly-top component. Tobacco plants that had recovered from curly top appeared to have a degree of resistance to infection by the vein-yellowing virus. Also, an appreciable percentage of tomato plants recovered following infection with a combination of the two viruses, whereas those infected with curly top alone invariably died. Characteristics and properties of the vein-yellowing virus indicate that it is related to the curly-top virus and that it probably originated from the latter by mutation.

The incidence of lemon shell bark in budlings from closely related sources. CALAVAN, E. C. Eureka lemon trees from some closely related lines differ greatly in their susceptibility to shell bark. Examination of 27,549 ten-year-old budlings from known scion sources shows that important differences may develop within two bud generations. Twenty per cent of the trees in one of five lines of second-generation budlings from a single original Blanchard Eureka source had shell bark. In a second line 0.3 per cent had shell bark. The other three lines were free. Lines of Allen Eureka budlings from closely related sources showed shell bark in from 0 to 76 per cent of the trees. The percentage of shell bark in trees of this strain appears to be related roughly to the amount of shell bark on the individual scion sources. Dodge Eureka lines had from 0 to 95 per cent shell bark. Some selections of Eureka lemon appear to be highly unstable with respect to shell-bark susceptibility. Extreme variation in shell-bark susceptibility is common among budlings from certain scion sources but rare among others. Shell-bark variants are often perpetuated by bud propagation.

A greenhouse method for testing tomatoes for resistance to curly top. CANNON, ORSON S., AND VICTOR WADDOUPS. One half of a 25-ft. by 25-ft. greenhouse section has been adapted for testing tomatoes for resistance to curly top by screening it from the other half of the section with 50-mesh cheesecloth and building a special concrete platform 2½ ft. high and containing 9 troughs 1 ft. apart, 2 in. deep, 20 in. wide, and 10 ft. long. Flats containing transplanted 10-day-old seedlings, with 6 sq. in. of space per seedling, are set in the troughs. After they have been subirrigated by filling the troughs with water and the excess water has been drained off, approximately 5 viruliferous leafhoppers per plant are released within the section. After one week, a similar number are released. First symptoms of curly top—twisting of leaves at the growing point and stunting of the plant—appear about 1 week after the first inoculation. Plants are classified after 1 month as healthy or diseased.

Laboratory studies of Western-X virus preparations. COCHRAN, GEORGE W. Preparations from Western-X virus-infected periwinkle plants and from virus-free periwinkle plants were subjected to ultracentrifuge fractionation procedures. Juices extracted from chopped frozen tissues were maintained at 0° to 1° C. during the fractionation. The ultraviolet absorption spectrum (from 2400 to 3000 Å) was obtained for all fractions using a Beckman D U spectrophotometer. No significant differences were found between any of the sedimented centrifuge fractions and their respective controls. The supernatant liquids obtained after the highest-speed centrifuge runs (214,500 G. for 40 min.) showed consistent differences. The preparations from the infected plants contained a material having a maximum of absorption at 2635 Å. This material did not appear to be present in the supernatants of the preparations made from virus-free plants.

Breaking patterns produced on peach flowers by the ring-spot virus. COCHRAN, L. C., AND R. L. MCCLAIN. Patterns of the general type characteristic of peach mosaic have been seen occasionally in flowers of several peach varieties affected with ring spot. In April, 1949, attention was called by peach mosaic control agencies to several orchards of the Rio Oso Gem variety, in which many trees had blossom breaking. On some trees up to 25 per cent of the flowers showed patterns. These trees were observed later in the season and none had leaf symptoms of either mosaic or ring spot. Transmission tests from these trees indicated that only ring-spot virus was present. The trees which showed blossom symptoms in 1949 showed similar symptoms again in 1950 and 1951. Blossom-breaking symptoms were produced on only a few flowers of trees infected experimentally with ring-spot virus. The variety used had large pink flowers but was not Rio Oso Gem, which has very large showy flowers that turn dark pink and appear to develop more symptoms than do blossoms of other varieties. Symptoms on some of the

petals appear identical with those caused by peach mosaic, but irregular and oak-leaf patterns unlike those of mosaic appeared on others. Generally, fewer of the flowers on the ring-spot-affected trees showed symptoms than did flowers on mosaic-affected trees.

Increased resistance to bean rust associated with water infiltration. COHEN, MORRIS. Detached Pinto bean leaves successively infiltrated with water, inoculated with uredospores of *Uromyces phaseoli*, incubated in a moist chamber (maintaining the translucent, water-soaked condition), allowed to recover (reentry of air into intercellular spaces with restoration of opaqueness), and then placed on 8 per cent sucrose solution have shown a definite decrease in pustule number and slightly delayed sporulation without important host injury. For infiltration, leaves were completely submerged in water in a vacuum desiccator, after which evacuation of the air to a nearly constant pressure of about 2 cm. Hg was followed by the sudden release of the vacuum, water thereby infiltrating the leaves. When leaves were allowed to recover from infiltration, either partially (scattered angular, opaque areas bounded by veins) or completely, by placing them in the laboratory air with petioles in water for varying lengths of time prior to inoculation and moist-chamber incubation, rust development in the recovered areas was apparently greater than in the twin noninfiltrated check leaves. The infiltrated condition of the leaf apparently did not interfere with the formation of appressoria, stomatal penetration, or formation of substomatal vesicles, but subsequent infection hyphae were rare. This accounts for the reduced infection.

Collar rot of tomato in California caused by Phytophthora capsici. CRITOPOULOS, P. D. A stem rot extending from soil level up to 15 cm. above has caused the death of isolated tomato plants near Stockton, California. The rotted portion is dark brown and there is no sharp demarcation with the adjacent healthy portion of the stem. The causal organism has been identified as *Phytophthora capsici* Leon. This isolate does not produce motile zoospores in water cultures with sterile water or with tap water. The swarm spores are discharged from the sporangium by action taking place within the sporangium. When discharged, they are fully differentiated, but variously lobed. The majority have no flagella; a few have only one. In all flagella observed there is a knob a short distance from their point of attachment. After discharge, zoospores drop slowly to the bottom of the plate or drift for a while on the surface or in the water. At the bottom of the plate the swarm spores assume in 5–15 min. a round form and may occasionally, by small jerky motions, approach each other or pull away. Two touching swarm spores unite within 10–20 min. to form one perfectly spherical body. The diameter of this body, tentatively called a zygote, decreases immediately after union, but it is always greater than that of the unpaired zoospores. It also has a darker protoplasmic content. The zygotes germinate by germ tube within 20 min. after their formation.

The Heterosporium disease of California poppy. DAVIS, LILY H. *Heterosporium eschscholtziae* Hark. has been known in California since 1884, but it did not cause economic loss until 1947 when it severely reduced yields in commercial seed fields. The disease may cause death of seedlings or lesions on leaves, stems, receptacle, calyptra, and fruit. The lesions are irregular brown to black spots which sporulate under moist conditions. In advanced stages the leaflets wither, and brown cankers at soil level cause the tap root to rot and shrivel. The mycelium may penetrate the capsule at its point of attachment, without inducing external symptoms, and ramify copiously through the interior of the fruit, killing young seeds and adhering to their surfaces or penetrating coats of mature seeds. In green seeds, the mycelium passes through the outer parenchymatous layers into the crystal layer, in many instances disorganizing it and preventing its formation, and then on into the embryo. After the cells of the crystal layer are mature, they apparently constitute a barrier through which only occasional strands of mycelium pass. Consequently in mature seeds the fungus is generally limited to the epidermis and to the layers of compressed parenchyma. The fungus is thus both externally and internally seed-borne. An efficient method of eliminating the fungus from the seed is a hot-water treatment at 125° F. for ½ hr.

Cladosporium rot of grapes in storage. DELP, CHARLES J., WM. B. HEWITT, AND K. E. NELSON. Cladosporium rot occurs periodically on grapes in storage in California. Symptoms in storage appear as well-defined black areas covered by the epidermis. After a short incubation period at higher temperatures the fungus becomes erumpent and olivaceous due to sporulation. Both injured and uninjured grapes were 100 per cent infected when sprayed with a spore suspension and stored in a moist chamber. The causal fungus was identified as *Cladosporium herbarum* (Pers.) Lk. Tests for growth of the fungus on potato-dextrose agar at different temperatures gave a gradual rise to an

optimum at about 24° C. and a rapid drop-off to no growth at 33° C. Infection developed at temperatures from 4° to 30° C. Curves for the degree of infection in Tokay and Emperor grapes were similar to curves for growth of the organism in culture. The peaks were about 21° C. for Tokay and 24° C. for Emperor, as based on arbitrary infection ratings. A close correlation was found this past season between rainfall during or shortly prior to harvest and the incidence of Cladosporium rot which developed in storage.

Mint rust (Puccinia menthae) epiphytotic in the Northwest. DIETZ, S. M., A. P. STEENLAND, AND C. E. HORNER. An epiphytotic of mint rust which developed in Oregon in 1949 resulted in a 25 per cent reduction in oil yield in Columbia County. This was the first serious outbreak of the disease in Oregon. By 1950 rust had spread to all of the mint-growing areas in the State. *Puccinia menthae* is an autoecious rust with all five spore forms functioning in Oregon. Teliospores, which may germinate soon after formation, overwinter on mint debris. Sporidia cause limited spread. Spread from aeciospores, which appear from February to April, is limited to a few feet. The main agents of dissemination are uredospores. Six applications of 10 per cent Fermate dust applied bi-weekly from May 17 to July 19 reduced leaf drop 46 per cent and number of uredosori 52 per cent. However, commercial use of Fermate is questionable since carbon disulphide was present in oil distilled from the treated plants. Studies are in progress to determine the best methods for removal of carbon disulphide from distilled oil. Methods of control which have given best results are such cultural practices as completely covering infected stubble by fall plowing, disking back furrows and head lands, and removal of volunteer mint plants surrounding the mint fields by spraying early in the spring with Dinitro followed by an application of 2,4-D.

Low-volume sprays for peach diseases. ENGLISH, HARLEY, AND E. E. WILSON. Fall applications of dilute and concentrated air-blast copper sprays for the control of Coryneum blight and leaf curl in peaches have been tested during the past two years. The more dilute sprays (100 to 400 gal./acre) were applied with sprayers that delivered either 27,000 or 40,000 cu. ft. of air per min.; the concentrated sprays (25 to 75 gal./acre) were applied with a machine that delivered 16,000 cu. ft. When equivalent amounts of copper were applied per acre, copper deposits obtained on twigs with the air-blast applications of basic copper sulfate plus an oil adhesive were as high as or higher than those obtained with the same materials or Bordeaux mixture applied with a hydraulic sprayer. Basic copper sulfate with a sticker appeared to adhere slightly better than Bordeaux mixture without an adhesive. The control of leaf curl and Coryneum blight obtained with air-blast applications of either basic copper sulfate or Bordeaux was as effective as that obtained with the same materials applied as dilute hydraulic sprays. Disease control obtained with air-blast applications at 25 gal. per acre was not improved by increasing the adhesive from 0.1 to 0.3 gal. per lb. of copper sulfate.

Control of the fungal complex causing transit rot of Vanda orchid blossoms. FEDER, WILLIAM A. Species of *Botrytis*, *Gloeosporium*, and *Alternaria* constitute an infective complex which often causes total destruction of Vanda orchid blossoms shipped from Hawaii to the continental United States. Affected blossoms become disfigured with brown specks or spots during shipment, and are usually broken down into a brown, rotted, watery mass upon arrival. Severe economic losses have been sustained during peak flower seasons. Inoculations with single-spore isolates of these fungi indicate that a single fungus or any combination of the three fungi is capable of causing the rot symptoms. The makeup of the complex is determined by the environment in which the flowers are raised, and symptom expression is largely controlled by the temperature at which the flowers are packed and shipped. Temperatures of 12°–16° C. favor *Botrytis*, 16°–20° C., *Gloeosporium*, and 20°–28° C., *Alternaria*. Active sporulation of all three fungi usually occurs after the flowers are thoroughly rotted. Excellent control was obtained by atomizing the flowers immediately before shipping, with a 1:2000 aqueous solution of 2-hydroxy-diphenyl formulated as Natriphene, or Dowicide A. Dehydroacetic acid was effective but slightly phytotoxic, and field treatment using various dithiocarbamates was ineffective.

Yellow bud blight, a foliar nematode disease of Vanda Miss Joaquim orchids. FEDER, WILLIAM A. Flowering spikes of Vanda Miss Joaquim plants grown for the export flower market are attacked by a foliar nematode, probably *Aphelenchoides ritzemabosi* or a closely related form. Affected flower buds turn yellow, then brown; they shrivel and fail to open. As high as 50 per cent of the flowering spikes in some fields have been affected. The nematode completes its life cycle within the buds. It is ap-

parently distributed by splashing wind-borne rain drops. The nematode species is morphologically similar to *Aphelenchoides ritzema-bosi* and has a thermal death point of 126° F. and a host range much like that of the chrysanthemum nematode. Nematodes were living between the bud scales and in the lumen of the buds, but in no case did they penetrate the tissues except to assume a feeding position. Excellent control was effected by spraying at weekly intervals with parathion at the rate of 2–4 lb. of 25 per cent wettable powder per 100 gal. of water plus B-1956. Laboratory tests indicated that kill was obtained through fumigant action of the parathion. A high degree of control was also obtained by the use of selenium as sodium selenate.

Soil fumigation against Verticillium albo-atrum. FERGUSON, JOHN, AND STEPHEN WILHELM. Experiments to determine direct fungicidal effects of soil fumigants on *Verticillium albo-atrum* included tests with the following chemicals each in at least three concentrations: allyl acetate, allyl alcohol, allyl bromide, allyl chloride, allyl formate, ammonium hydrosulfide, carbon disulfide, chlorobromopropene (Shell CBP-55), chloropicrin, ethylene dibromide, formaldehyde, isocapronic acid, methylol, nitroethane, nitromethane, o-chlorophenol, pentachlorethane, propylene bromide, propylene oxide, tetrachlorethane, tetrachlorethylene. Chemicals were injected into composted soil containing standardized Verticillium inoculum grown on tomato stem pieces placed at measured depths from 1.5 to 24 in. in soil 1 or 2 ft. deep and 2 sq. ft. in surface area in 32-gal. cans. Results were based on recovery in culture of *Verticillium* from the inoculum after treatment. Minimal dosage was expressed in cc. of chemical/sq. ft. of soil required to kill *Verticillium*, and can be converted to gal./acre by the factor of 11.5. Chloropicrin was effective against *Verticillium* at 2 cc./sq. ft., diffusing in lethal concentrations nearly equally well in all directions, allyl bromide at 2–3 cc./sq. ft. and Shell CBP-55 (55 per cent chlorobromopropene) at 4–5 cc./sq. ft., diffusing laterally and downward but not upward. The other chemicals named were not effective against *Verticillium* in these tests.

Occurrence and pathological effects of Stemphylium radicinum (M. D. & E.) Neerg. on carrots in California. GROGAN, R. G., AND WILLIAM C. SNYDER. The identities and pathological roles of *Stemphylium radicinum* (M. D. & E.) Neerg. and *Alternaria porii* (Ell.) Saw. f. *dauci* (Kühn pro var.) Neerg. have been confused frequently in the American literature. *A. porii* f. *dauci* is characterized by a long appendaged spore, and is best known as the cause of a foliage blight of carrot. *S. radicinum*, in contrast, has no spore appendage; it was described originally as the cause of a black root rot of carrot, and it has not been reported as causing foliage blight under natural conditions. In California during the past year *S. radicinum* has been found on four separate occasions causing foliage blight of carrot in the field. It is commonly seed-borne and can attack carrots in all stages of growth causing damping-off of seedlings, superficial spotting of unwounded roots, deep-seated black rot of wounded carrot roots, petiole and leaf blight, and blight of seed umbels.

The use of mosaic-free seed in controlling lettuce mosaic. GROGAN, R. G., J. E. WELCH, AND ROY BARDIN. From 1 to 3 per cent seed-borne lettuce mosaic virus was found in all commercial seed lots tested. Since seed-borne-virus-infected plants are scattered at random throughout commercial plantings, it was reasoned that they were the most important source of primary inoculum and that their elimination might effect control of the disease. Several pounds of mosaic-free seed of the varieties Imperial 615 and Great Lakes were produced by growing seedlings in an insect-free greenhouse and roguing out all seed-borne-infected plants prior to transplanting to a mosaic-free area. Samples of the mosaic-free seed were used to plant several test plots in commercial fields in the Salinas Valley area in the spring of 1951. Each plot was approximately 180 ft. square and was surrounded by commercial lettuce. Results from these tests showed a marked reduction in the percentage of mosaic in the areas planted with the disease-free seed, indicating that the use of such seed would be an effective method of controlling the disease.

Some responses of vines to sodium arsenite applied for black measles control. HEWITT, WM. B. Spot treatment of only vines with symptoms did not reduce the total number of diseased vines, but spraying all vines gave satisfactory control. Vines graded into healthy and five symptom classes before and after treatment showed that they shifted irregularly in the classes, i.e., from healthy into degrees of disease and the reverse. Shifts occurred in both sprayed and nonsprayed plots, but were greater from diseased to healthy in the former and the reverse in the latter. Spraying entire vines gave significantly better control than spraying only one side, and spraying after pruning was

better than spraying before pruning. Sodium arsenite (arsenic expressed as As_2O_3) sprayed on Thompson Seedless at concentrations of 1.5, 2.5, and 3.5 lb./100 gal. water reduced the percentage of measled vines from 18.2 to 9.7, 19.1 to 4.9, and 21.7 to 5.2, respectively; nonsprayed plots increased from 18.8 to 19.7 per cent. Successive annual sprays of sodium arsenite on Gamay vines reduced the yield 23 per cent after the second application, when compared with nonsprayed vines. Similar treatments to Muscat Frontignan and Thompson Seedless had no effect on yield even after three annual sprays.

Selection by laboratory assay of fungicides for field control of Botrytis leaf spot of gladiolus. HOLLOMAN, ARTHUR, JR., AND ROY A. YOUNG. The effectiveness of a number of chemicals in inhibiting germination of spores of *Botrytis gladiolorum* Timmermans was measured, using an agar-plate technique. Spore germination trials were conducted on plates of 2 per cent agar and 1 per cent dextrose to which quantities of chemicals had been added to give the desired concentrations. Five of the most promising fungicides were selected for field evaluation in 1949 and 1950. At the end of each season disease indexes were computed from leaf samples selected at random from each replication and graded into five classes on the basis of the amount of leaf area affected. Ferbam, nabam, Phygon XL, Puratized Agricultural Spray, and Carbide and Carbon's 341C are listed in order of their effectiveness in field control of Botrytis leaf spot. Although all of the materials tested caused a significant decrease in disease, ferbam (2 lb./100 gal.) was far superior to the other materials. The method of laboratory assay used in these trials proved to be a reliable criterion of field performance of the fungicides evaluated. The relative effectiveness of the chemicals in preventing spore germination was comparable to their relative effectiveness in preventing gladiolus leaf spot in the field.

Mosaic diseases of Ladino clover in California. HOUSTON, BYRON R., AND JOHN W. OSWALD. In 1950 Oswald reported a potato tuber necrosis disease caused by a strain of alfalfa mosaic virus from alfalfa. A similar relation was found to exist between Ladino clover and potato. Severe virus disease damage to some Ladino clover plantings has been observed in California. Inoculations onto Bountiful bean with juice from a number of diseased samples of Ladino clover, alfalfa, and potato indicated the presence of several viruses. The common virus present was the typical local lesion strain of alfalfa mosaic virus. Three types of systemic viruses were isolated. On Bountiful bean one type produced systemic symptoms of chlorotic mottle, downward rolling of leaflet margins, reddened nodes, and pod distortion followed by red discoloration. This group and the local lesion strains systemically infected potato, tobacco, and celery, producing symptoms typical of alfalfa mosaic virus. The other two systemic viruses appear to be confined to the legume family. On Bountiful bean one of these produces severe necrosis of growing points followed by extreme chlorotic mottle, if recovery occurs. The other virus produces mild symptoms of veinal chlorosis in the leaflets. The latter two viruses appear to have some relation to yellow bean mosaic.

The influence of nutrition upon tobacco mosaic virus-infected tomatoes. KENDRICK, J. B., JR., JOHN T. MIDDLETON, AND H. D. CHAPMAN. Marglobe tomatoes were inoculated with tobacco virus 1 after full expansion of the first leaf, and grown in sand cultures autoirrigated with solutions of high, medium, and low nitrogen, phosphorus, and potassium. All infected plants were uniformly reduced in fresh weight at 31 days. Compared with noninfected controls, infected plants under low-N nutrition showed minimum reduction in weight, while plants under high-N, -P, and -NPK nutrition showed maximum reduction. Weight of infected plants 37 days after inoculation was also less than noninfected plants but reduction was neither so uniform nor so great. Diseased plants predisposed at these nutrient levels before infection were not reduced in weight at 29 days so much as were infected nonpredisposed plants at 31 days. Increased plant growth after infection reduced the severity of the disease. Infected plants with low-N and -NPK nutrition had a smaller reduction in growth compared with comparable noninfected controls than infected plants with high-N or -NPK, although total growth was much less. Virus concentration was less with low-N and -NPK nutrients than with high-N and -NPK. Thus, severity of the disease is directly affected by virus concentration as well as the rate of recovery of infected plants under favorable nutrition. Fruit yield was materially reduced by infection. The reduction was minimized with medium-NPK, low-N, and high-K nutrition and accentuated with high-NPK, high-P, and low-K nutrients.

Histological studies of injury produced by four strains of curly-top virus on susceptible and resistant sugar beets. LACKEY, CHARLES F. Virulent virus strain 1 causes degeneration and necrosis of cells surrounding sieve tubes in root tips and severe necrosis of phloem tissue in petioles of the susceptible beet, SL 842. In the resistant beet, SL 86, the injury is similar but less in degree. Strain 2, a much less virulent strain, produces

only mild necrosis occasionally and some hypertrophy of pericycle cells adjacent to sieve tubes in root tips, and produces in petioles some hypertrophy and mild necrosis of phloem tissues. In the resistant beet only a few small necrotic areas appear in petioles and in cells adjacent to sieve tubes. Strain 6 does not produce so much vein distortion or veinlet clearing in beet leaves as do other curly-top strains and causes only small inconspicuous necrotic areas in petioles of susceptible and resistant beets; but it causes severe necrosis in many cells in sieve tube areas in root tips. Strain 22 induces a more marked disturbance in vascular bundles of petioles in susceptible and resistant beets than do any of the other strains. The disturbance consists of extreme hypertrophy of phloem cells with extensive necrosis and complete disorganization of bundles. Resistant beets have similar symptoms but to a lesser degree.

Botrytis on lilies. MACLEAN, NEIL ALLAN. *Botrytis elliptica* (Berk.) Cooke and *Botrytis cinerea* are two species of *Botrytis* that have been reported as occurring on *Lilium* sp. in North America. *B. elliptica* is a primary parasite and is capable of causing severe leaf spotting and at times bulb rotting. *B. cinerea* occurs on both foliage and bulbs, but as a secondary pathogen following a wound or injury. Recently *B. lilorum* Fujik. was isolated from bulbs of *Lilium auratum* Lindl. imported from Japan. This is believed to be the first report of this organism in North America. The infected bulbs had definite spotting and rotting of the outer and inner scales. There was evidence to suggest that in some cases the infection on the inner bulb scales had resulted from the organism spreading down the stem into the bulb.

Gladiolus smut. MACLEAN, NEIL ALLAN. Gladiolus smut (*Urocystis gladioli* (Req.) Smith) has recently been observed on cultivated gladiolus seedlings in the Bay area of California. The organism was found on the aboveground portions of the stem where it caused a blistering, shredding, and necrosis of stem and leaf tissues. Severely infected seedlings were killed. The characteristic smut balls of the organism were found in all infected tissues. Individual spores were 17–22 μ in diameter with 1–2 central cells surrounded by a single row of thin-walled, sterile cells.

Viruslike leaf malformation of spinach induced by 2,4-dichlorophenoxyacetic acid. MIDDLETON, JOHN T., AND J. B. KENDRICK, JR. An unusual leaf malformation was observed in a 3-month-old winter planting of Viroflay spinach. Affected plants were characterized by erect leaves with tubular petioles and peltate laminas. Affected leaves were of normal color, had no veinclearing, and were not distorted and straplike as is usual in plants treated with 2,4-dichlorophenoxyacetic acid. The terminal bud remained intact within the tubular petiole and was capable of growth and production of normal leaves. Healthy Viroflay spinach plants were incompletely wetted with a fine mist of 100 p.p.m. 2,4-dichlorophenoxyacetic acid. Cuplike leaf malformation developed one month later. Plants were placed in a fumigation chamber in which were hung 1.5 sq. ft. of paper toweling saturated with an ester of 2,4-D. Plants exposed continuously for 36 hr. had symptoms similar to those of sprayed plants. Plants exposed for two 15-hr. periods interrupted by sunlight for 8 hr. likewise developed symptoms indistinguishable from those of naturally affected plants and plants sprayed with 2,4-D. Since the naturally affected plants were not treated with 2,4-D and the nearest source of material was about 1 mile removed and treated 1 month before seeding spinach, it is concluded that the leaf malformation of spinach may be induced by vapors of 2,4-D.

Some histological aspects of Botrytis rot of table grapes. NELSON, K. E. The histological effects of *Botrytis cinerea* on the tissues of infected Tokay and Emperor grapes were studied to find an explanation for the characteristic symptoms of this rot. Infection through the cuticle took place by means of a small infection peg produced by an appressorium in contact with the surface of the berry. The host cells were separated to a depth of 6–10 layers by the enzymatic action of the subcuticular mycelium. Infected material stained with ruthenium red showed that the middle lamella had disappeared in the area of cell maceration. Thin slices of healthy tissue immersed in an enzymatic extract of the germ tubes of *Botrytis* showed the same narrow zone of cell separation evidenced in infected tissue. The mycelium was chiefly intercellular but often intracellular, causing lesions involving half the surface of the berry but only 1–2 mm. deep. The lesion site was indicated by a slight brownish discoloration on those berries with little or no red pigmentation. The skin over these areas could be ruptured with slight pressure and slipped off as a thin layer—hence the name "slipskin" commonly applied to this stage of the disease.

Histological studies of cherry fruits and pedicels of Western-X little-cherry in Idaho. NICHOLS, CARL W. Histological studies were made at the University of Idaho on little-

cherry and normal cherry fruits and pedicels. Diseased pedicels were shorter than normal and thicker than normal except near their base, where there was no difference in size. The cortex of diseased pedicels was also thicker except near the base. Pith diameter of diseased pedicels was normal at the apical and basal ends, but larger than normal in the center. Vascular bundle diameter was larger in the diseased pedicels throughout their length. Cell measurements showed that the small size of diseased fruits and pedicels was due to a lack of cell division rather than a lack of cell elongation. Wide epidermal cells, thick cuticle deposition, and a layer of injured cells just below the hypodermis caused the wrinkled appearance at the stylar end of the little-cherry fruits. Injured cells sometimes were found in the hypodermis and epidermis. Abscission layers were present in both, but functioned only in the normal cherry fruit.

Further observations on yellow leaf roll of peach. NYLAND, GEORGE. This virus disease has been observed for almost a full year under field conditions and in the greenhouse. Its lethal effects on limbs and entire trees has been verified. Many new cases have been observed this spring. The progress of the disease through the trees has been very rapid in most cases. This is especially true with vigorous 2- and 3-year-old replants which may become unprofitable in one year. No recovery of previously diseased limbs has been observed. In many respects the disease resembles buckskin on peach, which occurs in the same area. Among the differences are 1) the earlier appearance of symptoms of yellow leaf roll; 2) more rapid spread in the trees and in the orchards; 3) more rapid killing of limbs and trees; 4) greater persistence from one year to the next in diseased limbs; and 5) vein swelling in the field. Among the similarities are 1) characteristic leaf tattering due to abscission of laminal areas; 2) leaf casting; and 3) killing of sweet cherry on Mahaleb root. The similarities suggest a strain relationship between the causal agents of yellow leaf roll and buckskin.

A downy mildew of barley in California. OSWALD, JOHN W., AND BYRON R. HOUSTON. In 1929 Mackie reported a downy mildew occurring on barley in California. This disease has not been observed since. In the spring of 1951, a barley field near Davis had large areas in which all the plants were dead and the tissues were matted and shredded. Plants on the margins of these spots had severe yellow leaf streaking and were stunted, with many tillers killed outright. The shredded leaves, which consisted principally of fibrovascular tissues, were packed with oospores typical of the genus *Sclerospora*. Barley seedlings planted in diseased soil from this field were stunted and yellowed. Tissue examination demonstrated Sclerospora mycelium, oospores, and an abortive sporangial stage. Attempts to germinate the oospores (measuring 20 μ in diameter) have been unsuccessful. The only species of a downy mildew reported heretofore on barley has been *Sclerospora macrospora* (oospores 60 μ in diameter). This species does not cause tissue shredding. The fungus encountered on barley in 1951 more closely resembles *S. graminicola* (oospores 32 μ in diameter), which does induce severe shredding. This latter species, however, has been reported principally on hosts in the tribe Paniceae of the family Gramineae.

Similarities of symptoms produced by the viruses causing ring spot of peach and necrotic ring spot of sour and sweet cherry. PARKER, K. G., AND L. C. COCHRAN. Montmorency cherry trees and mazzard cherry seedlings grown in New York from seed of ringspot-free trees, provided by Karl D. Brase, were planted at Riverside, California, and inoculated with ring-spot virus from several sources. Separate lots of Montmorency inoculated with ring-spot virus, originating in peach orchards, or from Duke cherry collected in southern California, developed symptoms identical with those usually produced with inoculum from sour cherry affected with necrotic ring spot in New York. Montmorency inoculated with virus material from sweet cherry, collected in southern California, developed symptoms that were characterized by more severe necrosis than usual, but such as had sometimes been observed in New York and associated with necrotic ring spot. Mazzard seedlings inoculated by budding from these same virus sources and also from cultures originating in sweet cherry in Oregon produced symptoms similar to those obtained in New York with necrotic ring-spot virus from sour cherry.

A virus from mosaic-diseased Echinocystis in Oregon. PORTER, CLARK A., AND FRANK P. MCWHORTER. A virus isolated from the mottled leaves of wild cucumber (*Echinocystis oregana* Cogn.) was transmitted mechanically to National Pickling cucumber. The virus (O.S.C. isolate 606) produced yellow local lesions followed by conspicuous systemic yellow-patching of leaves and severe stunting. The virus was transmitted to Croft Easter lily, in which it produced fleck-streak symptoms. While transfer of the virus by mechanical means from cucumber to cucumber averaged above 90 per cent, no transfer was obtained to sugar beet, broad bean, Bountiful bean, Mt. Barker sub-

clover, California Wonder pepper, Burley and Turkish tobacco, Hearts of Gold muskmelon, Blue Hubbard squash, Klondike watermelon, or Pumila Dwarf zinnia. Failure of the 606 isolate to infect any of these plants indicates that it is not a strain of *Marmor cucumeris* H. Preliminary trials indicated that cucumber mosaic virus (O.S.C. strain 637) did not protect cucumber against infection by isolate 606. Isolate 606 was inactivated by a 10-min. exposure to 75° C.; it was infective at a dilution of 1: 1,000,000; and was infective after storage in macerated leaf tissue for 6 months at −20° C.

Hot-water treatment of gladiolus cormels. ROISTACHER, CHESTER N. Semidormant and nondormant gladiolus cormels were hot-water treated to determine the temperature causing a critical reduction in germination. Semidormant cormels of six varieties were treated for 30 min. at temperatures between 131° F. (55° C.) and 140° F. (60° C.). Cormels of these same six varieties were held at 40° F. for 3 weeks before hot-water treatment to render them nondormant. They were then treated at 133°, 135°, and 137° F. There was little varietal difference in the critical temperature in either type of experiment. Semidormant cormels survived treatment at 135° F. (56.8° C.), with a 40 per cent reduction in germination as compared with the checks. At 137° F. germination was reduced 75 per cent. The majority of nondormant cormels of the same variety did not survive the hot-water treatment at 133° F. or 135° F., which resulted in an 80 per cent and an 89 per cent reduction compared with the checks. Preliminary tests to determine the thermal death point of *Fusarium oxysporum* var. *gladioli* in gladiolus cormels indicate that the thermal death point of the fungus is close to the temperature that critically reduced germination of the cormels.

Stimulation and inhibition of conidial germination of Aspergillus niger. ROSENBERG, DAN Y. The conidia of *Aspergillus niger* do not germinate in distilled water; however, they will germinate on yeast extract media in 4–6 hr. at 30° C. A number of materials were added to yeast extract in varying concentration to determine the stimulatory or inhibitory effects. Juice from the inner fleshy scales of Utah Sweet Spanish onions inhibited germination, whereas extracts from the dry outer scales increased the rate and percentage of germination. In distilled water stimulation increased in direct proportion to the concentration of the onion extract. On yeast-extract media germination was completely inhibited with formaldehyde, 0.5 per cent, or 1.4 per cent Vancide 51 (a 30 per cent solution of sodium salts of dimethyl dithiocarbamic acid and 2 mercaptobenzothiazole). Inhibition to a lesser degree as to time of germination was shown with Hyamine 3258, trimethyl octadecyl ammonium pentachlorophenate. Only slight delays in germination were shown by n propyl mercaptan, 2.91 per cent; borax, 0.99 per cent; ferric dimethyl dithiocarbamate, 2.91 per cent; zinc ethylene bisdithiocarbamate, 2.91 per cent; and 8 hydroxy quinoline benzoate. On onion scales, however, formaldehyde was the only chemical that prevented normal germination. Conidial germination and infection of onion bulbs were favored by higher temperatures and inhibited by low temperature.

Fluorine injury to ponderosa pine. SHAW, CHARLES GARDNER, GEORGE W. FISCHER, DONALD F. ADAMS, AND MARK F. ADAMS. In addition to visible foliage damage, diameter growth of ponderosa pines in the damage area has been retarded since 1942. Damage is zonal, being most marked at a center coinciding with the industrial area north of Spokane, Washington. Damage cannot be correlated with climatic or parasitic factors. Chemical analyses of 5,000 needle samples collected during 1½ years over 180 sq. mi. reveal fluorides varying from 4 to 600 p.p.m. of the dry needle weight. Fluoride concentrations decreased with increased distance from the center of damage. Fluorides increase from year to year in pine needles until they fall off. Maximum concentrations of fluorides on the oldest needles in different zones were: 1-mi. radius, 620 p.p.m.; 3-mi. radius, 130 p.p.m.; 6–7-mi. radius, 40 p.p.m.; 9-mi. radius, 25 p.p.m. Air analyses revealed significant concentrations of fluoride in the atmosphere in the pine damage area. Highest concentrations (up to 351 p.p.b.) were found within 1 mi. of the center. At distances of 3.5–7 mi. up to 42 p.p.b. were present in the atmosphere. Injury to ponderosa pines and gladiolus has been prevented by spraying them with calcium oxide. Typical injury has been produced on pines and gladiolus in controlled experiments by fumigation for 24 hr. in an atmosphere containing 12 p.p.b. of hydrogen fluoride.

Use of heat to control sweet-potato black rot. SMITH, PAUL G., AND RALPH MICHAEL. Actively growing Petri-dish cultures of the sweet-potato black rot fungus, *Endoconidiophora fimbriata*, were incubated at 105°, 110°, 115°, and 120° F. and subcultured at 3-hr. intervals for the first 24 hr. The fungus remained alive for 24 hr. at 105° F. and for 6 hr. at 110° F. In subcultures taken at the 9-hr. interval at 110° F. and at the 3-hr. interval at 115° and 120° F., the organism failed to grow. Healthy sweet-potato roots were inoculated and, after 2 weeks, placed in a saturated atmosphere at 110° F. and at

120° F. Small lots of roots were removed at 3-hr. intervals up to 24 hr., the maximum treatment period. These lots were then held for 60 days at room temperature, the lesions inspected, and subcultures made to healthy roots. The black-rot fungus was killed in 9 hr. at 110° F. and in 3 hr. at 120° F. Roots with 3-month-old lesions on inoculated roots were handled similarly and subjected to three temperatures, 110°, 115°, and 120° F. Viable fungus was recovered after 24 hr. at 110° F., after 12 hr. at 115° F., and after 3 hr. at 120° F. Roots subjected to 110° F. for 24 hr. showed no evidence of heat injury nor loss of flavor. After 12 hr. at 115° F. and after 3 hr. at 120° F., heat injury to the roots was severe.

Field crop rotations for control of Phymatotrichum root rot in irrigated lands. STREETS, R. B. Profitable yields are being secured on root-rot-infested irrigated land using rotations with cotton or flax as the principal cash crop. Experiments have run 7 years. Control plots had almost 100 per cent dead cotton plants in third, fourth, and fifth years, and yield declined from 1800 lb. of seed cotton the first year to 600 the fourth year. A 5-year rotation with flax as the cash crop, using early guar as a seed crop, or guar or sesbania as green manure crops, has reduced severe root rot to a trace, 2 per cent, and 4 per cent, respectively, in the second crop of cotton grown after the 5-year rotation. A profitable crop of cotton was grown each year on root-rot-infested land by plowing under a winter crop of Papago peas and planting an early maturing variety of cotton such as Paula C or one of the new Arizona varieties, X33 and X44, developed by Presley. Commercial nitrogen and sulphur were used in this and the following rotation. Yield was 2656 lb. per acre with 1.7 per cent root rot in 1950. A 2-year rotation (barley, guar for seed, cotton) gives better yields (3335 lb. per acre in 1950), but does not control root rot so well (13.3 per cent).

The Phytophthora root rots of Chamaecyparis lawsoniana Parl. TORGESON, D. C. *Phytophthora lateralis* Tucker & Milbrath has previously been reported as causing a root rot of varieties of Lawson cypress, *Chamaecyparis lawsoniana* Parl., in Oregon. During the past two years *P. cinnamomi* Rands has caused a root rot in several plantings of varieties of Lawson cypress. Symptoms caused by the two fungi were identical. *P. cinnamomi* has also caused a root rot in plantings of Irish yew, *Taxus baccata* L. var. *fastigiata* Loud. These two species of Phytophthora are readily distinguished by differences in their morphological characteristics, temperature requirements, and rate of growth. *P. lateralis* appears to be much more widely distributed in Oregon than *P. cinnamomi*, although the presence of the latter is perhaps more serious because of its much wider host range. The isolates of *P. cinnamomi* from Irish yew and Lawson cypress compare with isolates from other hosts in the United States. Experimentally the Oregon isolates of *P. cinnamomi* have caused a root rot of *Rhododrendron ponticum* L., *Castanea dentata* Borkh., and *Taxus cuspidata* Sieb. & Zucc.

The inheritance of resistance to tobacco mosaic virus in interspecific tomato crosses. WATSON, R. D., AND E. C. HEINRICH. The inheritance of resistance to tobacco mosaic virus was studied in crosses of *Lycopersicon esculentum* var. Sioux × *L. hirsutum* tomato. One hundred per cent of the plants of Sioux, of the F_1 hybrids, and of the backcrosses to Sioux had evident symptoms. A ratio of three symptomless plants to one plant with symptoms was obtained in the backcross to *L. hirsutum*. This indicated at least two factors for symptom expression. A good correlation was obtained between the symptom expression and the virus concentration as tested by the necrotic lesion method. This correlation indicated the severity of the symptom expression to be a measure of resistance. All the plants tested, both parent and progeny, carried the virus. An unequal influence of the factors for resistance was indicated. The narrow range of average virus concentration between individual F_1 hybrid plants showed the parent species to be fairly homozygous for the factors of resistance or susceptibility. These studies indicate that only a few factor pairs were involved in the inheritance of resistance in these crosses. The necrotic lesion test is a useful tool in selecting for resistance to tobacco mosaic virus in tomato.

Is Verticillium albo-atrum a soil invader or a soil inhabitant? WILHELM, STEPHEN. Fungus pathogens may inhabit soils, i.e., grow saprophytically in competition with the general soil microflora, or invade soils, their saprophytic existence restricted to a precarious tenancy of plant material invaded while pathogenic, persistence depending upon the durability of resting structures. Evidence obtained from studies of *Verticillium albo-atrum* suggests that it is a soil invader. Tomato can bring about a rapid increase of *Verticillium* in soil. *V. albo-atrum* was not found as a component of the root surface or rhizosphere microflora of tomato, which flora consisted mainly of *Pythium ulti-*

mum, Rhizoctonia solani, Fusarium roseum, F. oxysporum, Stemphylium, and *Mucor* spp. When three natural soils were used and *Verticillium* was introduced as spores on sterilized tomato stem pieces or as sclerotia formed on the pieces, *Verticillium* in no instance grew through the soil nor colonized a ring of tomato stem pieces 1 cm. distant in 6 weeks. Only at a low temperature (16° C.) did the spore inoculum consistently colonize the inoculum pieces. In sterilized soil *Verticillium* grew readily. Of the soil fungi isolated and permitted to colonize the soil singly, *Gliocladium, Chaetomium, Stachybotrys,* and *Myrothecium* spp. killed or checked growth of *Verticillium,* whereas *Trichoderma, Fusarium,* and *Mucor* spp. produced little or no unfavorable effect.

Gomphrena globosa, a local-lesion indicator plant for the carnation mosaic virus. WRIGHT, CHARLES M. In preliminary studies with a sap-transmissible mosaic virus of carnation, attempts were made to find a local-lesion plant that could be used for indexing carnation plants. The globe amaranth, *Gomphrena globosa,* developed discrete reddish brown local lesions approximately 5 days following inoculation with sap from plants of mosaic-infected carnation and *Dianthus barbata.* The virus was recovered from inoculated tomato plants displaying a slight leaf rolling and dwarfing. The virus was not inactivated at 60° C., the highest temperature used, and was recovered after aging *in vitro* for 36 hr. Presumably the virus producing the lesions on the globe amaranth was the carnation mosaic virus, but further studies should be made to determine this.

The phosphate effect in virus inoculations. YARWOOD, C. E. Water solutions of K_2HPO_4 were injurious to leaves and the amount of injury depended on concentration of K_2HPO_4, time of exposure, species and age of leaves, method of application, and environment. When 1 per cent K_2HPO_4 was added to the inoculum of tobacco mosaic virus (TMV), tobacco necrosis virus, and seven other viruses, greater numbers of local lesions resulted on bean than when no phosphate was used. On Pinto bean inoculated with TMV the ratio of the number of lesions formed without supplement, with 1 per cent K_2HPO_4 added to the inoculum, with carborundum dusted on the leaves before inoculation, and with both K_2HPO_4 and carborundum, was 1: 20: 165: 2860, respectively. The ratio of the amount of inoculum to produce one lesion per leaf under these same conditions of inoculation was about 100,000: 1,000: 100: 1 respectively. Treatment of bean leaves with phosphate before or after inoculation with TMV increased infection in some cases, but the greatest response resulted when the phosphate was added to and applied with the inoculum. Addition of phosphate to the TMV inoculum applied to carborundum-dusted leaves of *Nicotiana glutinosa* caused increased numbers of lesions on the upper leaves but decreased numbers on the lower leaves.

Temperatures of diseased leaves. YARWOOD, C. E. Leaf temperatures were measured to 0.01° C. with mercury thermometers in stoppered vacuum cups, which, when containing 150 cc. of warm water, lost heat at the rate of 11 calories per hr. for each centigrade degree of temperature difference between the inside and the outside of the vacuum cups. For measurements of leaf temperature, 10 gm. of leaves were wrapped snugly around the thermometer bulbs and the leaves in vacuum cups were placed at 25° C. Temperatures of an empty vacuum cup, of one containing healthy leaves, and of one containing diseased leaves were observed simultaneously. Temperatures of various healthy leaves ranged from 0.32° to 1.74° C. higher than air temperature. When a high proportion of the diseased tissue was living, the temperature of diseased leaves was always higher than that of healthy ones. When the difference in temperature between diseased and healthy leaves is expressed as a percentage of the difference in temperature between corresponding healthy tissues and the air, the following percentage increases in temperature due to disease were calculated: bean rust, 206; bean powdery mildew, 37; lettuce downy mildew, 327; bean anthracnose, 146; tobacco mosaic, 52; white clover mosaic virus on bean, 7; and cabbage black ring virus on *Nicotiana glutinosa,* 14.

A method of treating freshly cut seed-pieces on tuber-unit potato planters. YOUNG, ROY A., AND J. A. MILBRATH. Potato seed-piece decay, caused by Fusarium and Pythium spp., annually results in reductions in stand ranging from a trace to losses that necessitate replanting. Poor stands frequently occur in fields planted with tuber-unit potato planters, on which potatoes are cut and planted immediately. To prevent such losses there has been need for a satisfactory method of treating seed-pieces in the interval between cutting and planting. Excellent results were obtained in preliminary trials with a tuber-unit planter on which seed-pieces were cut on a vertical rotating wheel of knives and passed through a fungicide bath before being planted. Treatment with Phygon (1 lb./10 gal.) and Semesan Bel (1 lb./7½ gal.) resulted in marked yield in-

creases. Excellent results have been obtained with Phygon in all seed-piece treatment trials. Phygon favors suberization and provides protection against *Fusarium solani* f. *radicicola* (Wr.) Snyder & Hansen, and *F. roseum* (Link) Snyder & Hansen. Mercury compounds appear promising for treatment of cut seed-pieces if injury can be avoided by planting soon after treating. A tuber-unit potato planter adapted for seed-piece treatment should be a great asset to seed-potato growers and should also facilitate the use of mercury compounds on cut potato seed-pieces.

Fungicides for the control of avocado fruit rot. ZENTMYER, GEORGE A., AND L. J. KLOTZ. *Botryosphaeria ribis* (*Dothiorella* sp.) causes a severe rot of avocado fruit from coastal southern California plantings. The fungus enters while fruit are still on the tree but rot develops only when the fruit begins to soften, by which time fruit has reached the market or consumer. Spray trials over the past 4 years have shown that the disease can be controlled by two applications of Bordeaux, Crag Potato Fungicide 658, Bioquin 1, or Parzate. In trials in 1950–1951, with randomized treatments replicated 5 times, the percentages of fruit developing rot in various treatments following picking in January and in March respectively were: Bordeaux (two sprays, Sept. 20 and Nov. 1), 4 and 10; 658 (two sprays), 2 and 11; Bioquin 1 (two sprays), 4 and 40; 658 (one spray, Sept. 20), 12 and 38; and unsprayed, 62 and 83. In general, 658 is the most satisfactory material because of the degree of control and the inconspicuous residue. Attempts to control the disease by killing the fungus after it has entered the fruit have been uniformly unsuccessful. Treatments included: paper wrappers impregnated with fungicides (azochloramide, alpha terpineol, 8-hydroxyquinoline sulfate); fungicidal dips (alpha terpineol, tetramethylthiuram-disulfide, 8-hydroxyquinoline benzoate, Dithane D14, Puratized Agricultural Spray); fumigation with ethylene oxide, propylene oxide, hydrogen sulfide, nitrogen trichloride; and heat treatments (hot water and hot, humid air).

PHYTOPATHOLOGICAL NOTE

Report on Indicator Plants for Chrysanthemum Stunt Virus and on a Previously Unreported Chrysanthemum Virus. JOHN R. KELLER. Investigations on chrysanthemum stunt virus at Cornell University[1] have resulted in the discovery of a previously unreported masked virus in the variety Blanche. It also was found that Blanche (infected with the new virus) and Mistletoe are excellent indicator varieties for chrysanthemum stunt virus.

For some years a systemic disease, termed "measles" by commercial growers, has caused numerous conspicuous white leaf spots about $\frac{1}{8}$ in. in diameter in the variety Mistletoe (Fig. 1). Evidence that the disease is caused by the chrysanthemum stunt virus was obtained by cross grafting Mistletoe plants with measles symptoms to healthy plants of the varieties Vibrant, Sea Gull, or Blazing Gold: the symptoms produced in these varieties were identical with those characteristic of chrysanthemum stunt. In reciprocal grafts, healthy Mistletoe plants inoculated with chrysanthemum stunt virus developed typical measles. Similar results were obtained in cross-inoculation experiments using juice. Since the measles-type symptom is very distinctive the variety Mistletoe has proved useful for diagnosis of stunt by either graft or juice inoculation.

A previously unreported virus was found together with chrysanthemum stunt virus in plants of the variety Blanche, showing symptoms of the disease known as "crinkle." This disease has been known for several years in the variety Blanche and is characterized by very severe distortion of the leaves, accompanied by small white flecks and streaks over the leaf surface (Fig. 1). Some supposedly healthy and crinkle-infected plants of this variety were obtained and included in the above cross-graft experiments. A new symptom expression was noted in all plants grafted with the variety Blanche. In combinations involving supposedly healthy Blanche and healthy plants of other varieties, severe leaf distortion and yellow vein-banding resulted in Mistletoe and Blazing Gold, whereas only yellow vein-banding occurred in Vibrant and Sea Gull. In all grafts between "healthy" Blanche and stunt-infected plants of the other four varieties, the severe crinkle disease resulted in Blanche. In the other four varieties a more severe reaction than that produced by the stunt virus alone occurred. It was apparent that a complex of two viruses was involved in the crinkle disease—chrysanthemum stunt virus and a hitherto unreported virus which was present, but symptomless, in the supposedly healthy Blanche. This new virus has been termed chrysanthemum virus Q. The lack of cross-protection in numerous experiments involving both graft and juice inoculation and the fact that virus Q does not appear to be sap-transmissible to

[1] Keller, J. R. Chrysanthemum stunt. Florists' Review. 107 (2762): 37–38. November 2, 1950.

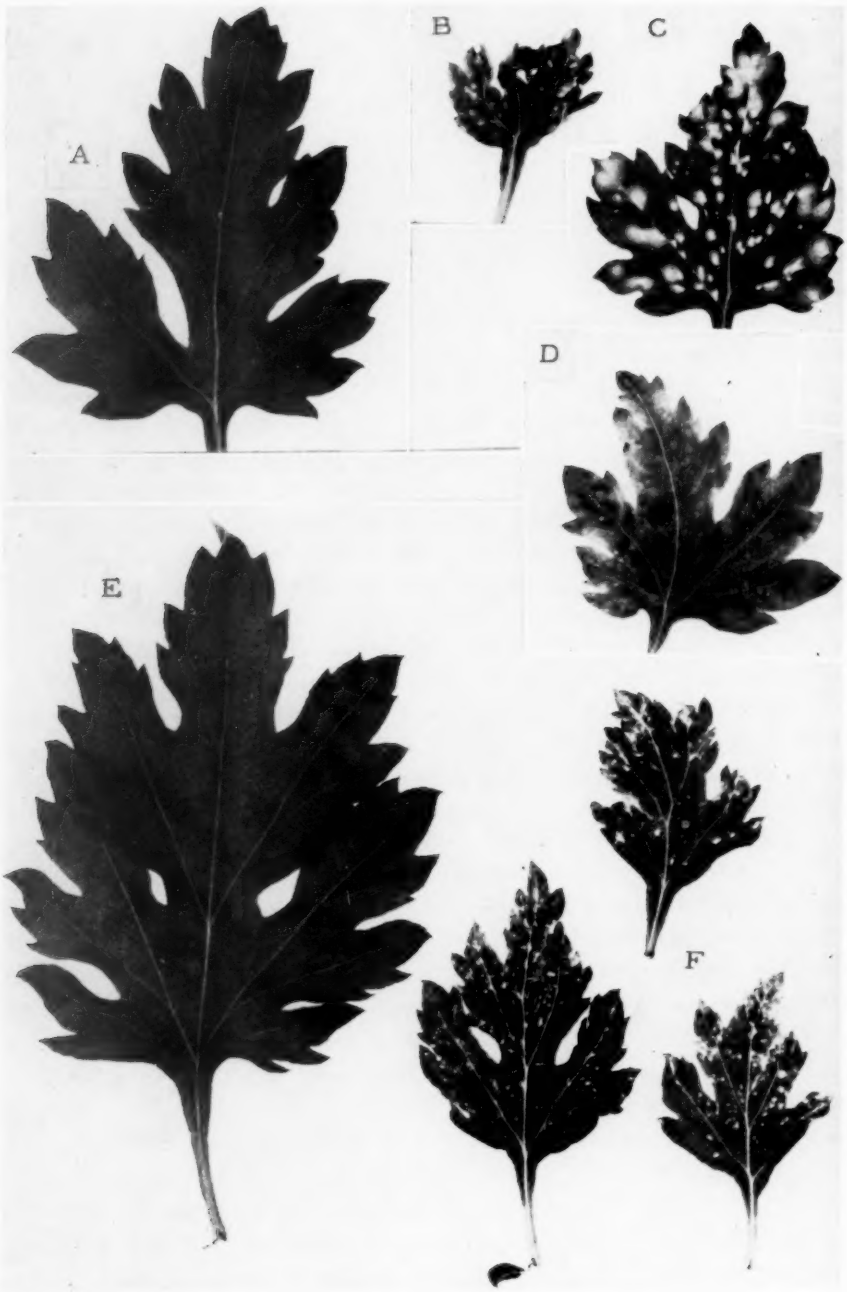

FIG. 1. Leaves from chrysanthemum plants; A–D, the variety Mistletoe: A, from healthy plant; and from plants infected with (B) stunt ("measles") and virus Q, (C) stunt, and (D) virus Q. E and F, from the variety Blanche: E, from plant infected with virus Q; F, three leaves with distortion caused by presence of "crinkle" complex.

chrysanthemum indicate that virus Q is not related to the chrysanthemum stunt virus.[2]

Negative results of tissue plantings and microscopic examinations showed that neither bacteria nor fungi are involved in the diseases here considered.

Plants of the variety Blanche infected with virus Q have to date been the most valuable indicator plants available for stunt diagnosis. The positive reaction indicated by the appearance of crinkle symptoms when stunt is superimposed on virus Q has been very reliable and easily recognized at any time of the year.

The use of the chrysanthemum as an indicator plant for the stunt virus is necessitated by the fact that no other satisfactory test plant has been found. In the fall of 1949, nearly 90 different plant species included in 27 families were inoculated with the stunt virus, but not one gave a reliable reaction.—Department of Plant Pathology, Cornell University, Ithaca, New York.

[2] The possibility is being investigated that virus Q was involved in the stunt-mottle disease reported by Welsh (Welsh, M. F. Stunt-mottle virus disease of chrysanthemums. Sci. Agr. 28: 422. 1948).

ERRATUM

In the article, "The insect vector relationships of the filaree red-leaf virus, with special reference to a latent-period difference between nymphs and adults in Macrosiphum geranicola (Lambers)," by Chris W. Anderson, August 1951 issue (Vol. 41: 699–708), the printers transposed the four lines following table 3, page 705. These four lines should follow the last line on the page, reading:

"The results (Table 3) add *Macrosiphum pelargonii*, *Aphis rumicis*, and *Rhopalosiphum prunifoliae* to the list of vectors, and *Aphis gossypii* to the nonvectors, reported by Frazier. Tests with the other species confirm the conclusions reached by Frazier, but higher percentages of transmission (for equal numbers of aphids per test plant) were generally obtained in the present work. . . ."

If the subscriber will encircle with pencil the four lines of type following table 3 and indicate transposition to the foot of the page, the text will be clear.